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(54) **Amino-hydroxy-methyl-isoxazole-propionate binding human glutamate receptors.**

(57) Described herein are isolated polynucleotides which code for a family of AMPA-type human CNS receptors. The receptors are characterized structurally and the construction and use of cell lines expressing these receptors are disclosed.

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FIG. 1G

FIG. 1H

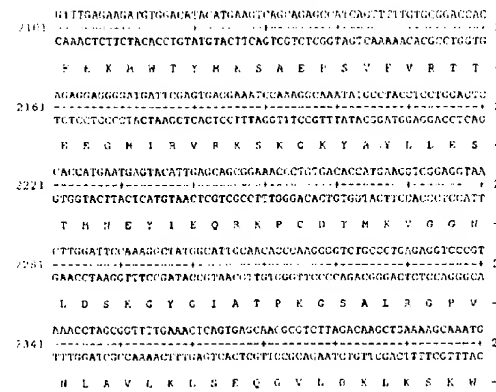


FIG. 1I

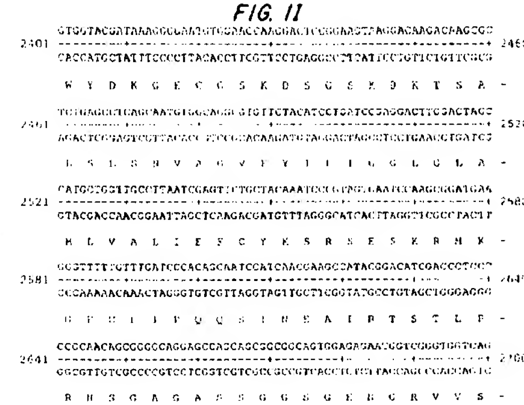


FIG. 1J

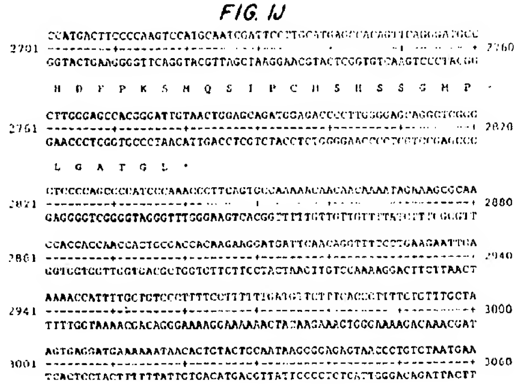
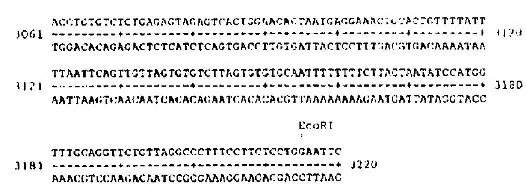


FIG. 1K



Background of the Invention

Field of the Invention

This invention is concerned with applications of recombinant DNA technology in the field of neurobiology. More particularly, the invention relates to the cloning and expression of DNA coding for excitatory amino acid (EAA) receptors, especially human EAA receptors.

Background of the Invention

In the mammalian central nervous system (CNS), the transmission of nerve impulses is controlled by the interaction between a neurotransmitter substance released by the "sending" neuron which then binds to a surface receptor on the "receiving" neuron to cause excitation thereof. L-glutamate is the most abundant neurotransmitter in the CNS, and mediates the major excitatory pathway in vertebrates. Glutamate is therefore referred to as an excitatory amino acid (EAA) and the receptors which respond to it are variously referred to as glutamate receptors, or more commonly as EAA receptors.

Using tissues isolated from mammalian brain, and various synthetic EAA receptor agonists, knowledge of EAA receptor pharmacology has been refined somewhat. Members of the EAA receptor family are now grouped into three main types based on differential binding to such agonists. One type of EAA receptor, which in addition to glutamate also binds the agonist NMDA (N-methyl-D-aspartate), is referred to as the NMDA type of EAA receptor. Two other glutamate-binding types of EAA receptor, which do not bind NMDA, are named according to their preference for binding with two other EAA receptor agonists, namely AMPA (α-amino-3-hydroxy-5-methyl-isoxazole-4-propionate), and kainate. Particularly, receptors which bind glutamate but not NMDA, and which bind with greater affinity to kainate than to AMPA, are referred to as kainate type EAA receptors. Similarly, those EAA receptors which bind glutamate but not NMDA, and which bind AMPA with greater affinity than kainate are referred to as AMPA type EAA receptors.

The glutamate-binding EAA receptor family is of great physiological and medical importance. Glutamate is involved in many aspects of long-term potentiation (learning and memory), in the development of synaptic plasticity, in epileptic seizures, in neuronal damage caused by ischemia following stroke or other hypoxic events, as well as in other forms of neurodegenerative processes. However, the development of therapeutics which modulate these processes has been very difficult, due to the lack of any homogeneous source of receptor material with which to discover selectively binding drug molecules, which interact specifically at the interface of the EAA receptor. The brain derived tissues currently used to screen candidate drugs are heterogeneous receptor sources, possessing on their surface many receptor types which interfere with studies of the EAA receptor/ligand interface of interest. The search for human therapeutics is further complicated by the limited availability of brain tissue of human origin. It would therefore be desirable to obtain cells that are genetically engineered to produce only the receptor of interest. With cell lines expressing cloned receptor genes, a substrate which is homogeneous for the desired receptor is provided, for drug screening programs.

Recently, genes encoding substituent polypeptides of EAA receptors from non-human sources, principally rat, have been discovered. Hollmann et al., Nature 342: 643, 1989 described the isolation from rat of a gene referred to originally as GluR-K1 (but now called simply GluR1). This gene encodes a member of the rat EAA receptor family, and was originally suspected as being of the kainate type. Subsequent studies by Keinanen et al., Science 249: 556, 1990, showed, again in rat, that a gene called GluR-A, which was identical to the previously isolated GluR1, in fact encodes a receptor not of the kainate type, but rather of the AMPA type. These two groups of researchers have since reported as many as five related genes isolated from rat sources. Boulter et al., Science 249: 1033, 1990, revealed that, in addition to GluR1, the rat contains 3 other related genes, which they called GluR2, GluR3, and GluR4, and Bettler et al., Neuron 5: 583. 1990 described GluR5. Keinanen et al., supra, described genes called GluR-A, GluR-B, GluR-C and GluR-D which correspond precisely to GluR1, GluR2, GluR3 and GluR4 respectively. Sommer et al., Science 249: 1580, 1990 also showed, for GluR-A, GluR-B, GluR-C and GluR-D two alternatively spliced forms for each gene. These authors, as well as Monyer et al., Neuron 6: 799, 1991 were able to show that the differently spliced versions of these genes are differentially expressed in the rat brain.

There has emerged from these molecular cloning advances a better understanding of the structural features of EAA receptors and their subunits, as they exist in the rat brain. According to the current model of EAA receptor structure, each is heteromeric in structure, consisting of individual membrane-anchored subunits, each having four transmembrane regions, and extracellular domains that dictate ligand binding properties to some extent and contribute to the ion-gating function served by the receptor complex. Keinanen et al, supra, have shown for example that each subunit of the rat GluR receptor, including those designated GluR-A, GluR-

B, GluR-C and GluR-D, display cation channel activity gated by glutamate, by AMPA and by kainate, in their unitary state. When expressed in combination however, for example GluR-A in combination with GluR-B, gated ion channels with notably larger currents are produced by the host mammalian cells.

In the search for therapeutics useful to treat CNS disorders in humans, it is highly desirable of course to provide a screen for candidate compounds that is more representative of the human situation than is possible with the rat receptors isolated to date. It is particularly desirable to provide cloned genes coding for human receptors, and cell lines expressing those genes, in order to generate a proper screen for human therapeutic compounds. These, accordingly, are objects of the present invention.

Summary of the Invention

The present invention provides isolated polynucleotides that code for a family of AMPA-binding human EAA receptors, herein referred to as "GluR receptors". By providing polynucleotides that code specifically for CNS receptors native to humans, the present invention provides means for evaluating the human nervous system, and particularly for assessing potentially therapeutic interactions between the AMPA-binding human EAA receptors and selected natural and synthetic ligands.

In one of its aspects, the present invention provides an isolated polynucleotide that codes for an EAA receptor belonging to the human GluR family. Alternatively, the polynucleotide may code for an AMPA-binding fragment of a human GluR receptor, or for an AMPA-binding variant of a human GluR receptor. According to specific embodiments of the present invention, the isolated polynucleotide encodes the human GluR1B receptor, the amino acid sequence of which is identified in Figure 1 (SEQ ID NO: 2), the human GluR2B receptor the amino acid sequence of which is identified in Figure 2 (SEQ ID NO: 4), and the human GluR3A receptor, the amino acid sequence of which is identified in Figure 3 (SEQ ID NO: 6). According to another embodiment of the invention, the polynucleotide encodes an AMPA-binding variant of the human GluR receptor. One such variant is identified herein as the human GluR3B receptor, the amino acid sequence of which is identified in Figure 4 (SEQ ID NO: 8). In various specific embodiments of the present invention, the polynucleotide consists of DNA e.g. cDNA, or of RNA e.g. messenger RNA. In other embodiments of the present invention, the polynucleotide may be coupled to a reporter molecule, such as a radioactive label, for use in autoradiographic studies of human GluR receptor tissue distribution. In further embodiments of the present invention, fragments of the polynucleotides of the invention, including radiolabelled versions thereof, may be employed either as probes for detection of glutamate receptor-encoding polynucleotides, as primers appropriate for amplifying such polynucleotides present in a biological specimen, or as templates for expression of a GluR receptor or AMPA-binding fragments or variants thereof.

According to another aspect of the present invention, there is provided a cellular host that produces an AMPA-type human glutamate receptor, and is characterized by the incorporation therein of a polynucleotide of the present invention. In embodiments of the present invention, the polynucleotide is a DNA molecule and is incorporated for expression and secretion in the cellular host, to yield, upon culturing, a functional, membrane-bound human GluR receptor. In other embodiments of the present invention, the polynucleotide is an RNA molecule which is introduced into the cellular host to yield a human GluR receptor as a functional, membrane-bound product of translation.

According to another aspect of the invention, there is provided a process for obtaining a substantially homogeneous source of a human EAA receptor useful for performing ligand binding assays, which comprises the steps of culturing a genetically engineered cellular host of the invention, and then recovering the cultured cells. Optionally, the cultured cells may be treated to obtain membrane preparations thereof, for use in the ligand binding assays.

According to another aspect of the present invention, there is provided a method for assaying interaction between a test ligand and a human EAA receptor, comprising the steps of incubating the test ligand under appropriate conditions with a human GluR receptor source, i.e., a cellular host of the invention or a membrane-preparation derived therefrom, and then determining the extent or result of binding between the substance and the receptor source.

These and other aspects of the invention are now described in greater detail with reference to the accompanying drawings, in which:

Brief Description of the Drawings

Figure 1 provides a DNA sequence coding for the human GluR1B receptor, and the amino acid sequence thereof (SEQ ID NOS: 1 and 2);

Figure 2 provides a DNA sequence coding for the human GluR2B receptor, and the amino acid sequence

thereof (SEQ ID NOS: 3 and 4);

Figure 3 provides a DNA sequence coding for the human GluR3A receptor, and the amino acid sequence thereof (SEQ ID NOS: 5 and 6);

Figure 4 provides a DNA sequence coding for the human GluR3B receptor, and the amino acid sequence thereof (SEQ ID NOS: 7 and 8);

Figure 5 provides the amino acid sequence of the human GluR3A receptor (SEQ ID NO: 9) and the human GluR3B receptor (SEQ ID NO: 10) in a region of dissimilarity;

Figure 6 depicts the strategy employed in cloning the human GluR3A receptor-encoding DNA illustrated in Figure 3;

Figure 7 depicts the strategy employed in cloning the human GluR3B receptor-encoding DNA illustrated in Figure 4;

Figure 8 depicts the strategy employed in generating recombinant DNA expression constructs incorporating the GluR3A receptor-encoding DNA;

Figure 9 depicts the strategy employed in generating recombinant DNA expression constructs incorporating the GluR1B receptor-encoding DNA (SEQ ID NOS: 11 and 12 are also shown in this figure);

Figure 10 depicts the strategy employed in cloning the human GluR2B receptor-encoding DNA illustrated in Figure 2;

Figure 11 depicts the strategy employed in generating recombinant DNA expression constructs incorporating the GluR2B receptor-encoding DNA;

Figure 12 illustrates the AMPA-binding property of the human GluR1B receptor;

Figure 13 illustrates the AMPA-binding property of the human GluR2B receptor;

Figure 14 illustrates the AMPA-binding property of the human GluR3A receptor;

Figures 15 & 16 illustrate a Scatchard analysis of human GluR1B and GluR2B receptor AMPA binding; and

Figure 17 graphically shows AMPA competition binding data for the GluR2B receptor.

Detailed Description of the Preferred Embodiments

The invention relates to human CNS receptors of the AMPA-binding type, and is directed more particularly to novel receptors belonging to a family herein referred to as "GluR receptors", and provides isolated polynucleotides that code for such receptors. The term "isolated" is used herein with reference to intact polynucleotides that are generally less than about 4,000 nucleotides in length and which are otherwise isolated from DNA coding for other human proteins.

As used herein, the term "GluR receptors" is intended to embrace the human GluR1B, GluR2B and GluR3A receptors, AMPA-binding variants related thereto, as well as AMPA-binding fragments of the GluR1B, GluR2B and GluR3A receptors. Receptor variants within the scope of the present invention are functional variants of a parent receptor, i.e., one of GluR1B, GluR2B, GluR3A and GluR3B, which include conservative amino acid substitutions.

The term "AMPA-binding", as used herein with respect to receptors, and variants and fragments thereof, refers to a ligand binding profile which reveals glutamate binding and relative greater binding affinity for AMPA than for either glutamate, kainate or NMDA, as determined using assays of conventional design, such as the assays herein described.

In the present specification, an AMPA-binding receptor is said to be "functional" if a cellular host producing it exhibits *de novo* channel activity when exposed appropriately to AMPA, as determined by the established electrophysiological assays described for example by Hollmann et al., *supra,* or by any other assay appropriate for detecting conductance across a cell membrane.

Members of the human GluR family of the invention possess structural features characteristic of the EAA receptors in general, including extracellular N- and C-terminal regions, as well as four internal hydrophobic domains which serve to anchor the receptor within the cell surface membrane.

More specifically, the GluR1B receptor is a protein characterized structurally as a single polypeptide chain that is produced initially in precursor form bearing an 18 amino acid residue N-terminal signal peptide, and is transported to the cell surface in mature form, lacking the signal peptide and consisting of 888 amino acids arranged in the sequence illustrated, by single letter code, in Figure 1 (SEQ ID NOS: 1 and 2). Unless otherwise stated, the term human GluR receptor, either generally or with reference to a particular member of the receptor family, refers to the mature form of the receptor. Thus, the amino acid residues of these receptors are numbered in Figures 1-4 with reference to the mature protein sequence. With respect to structural domains of the GluR1B receptor, hydropathy analysis reveals four putative transmembrane domains, one spanning residues 521-540 inclusive (TM-1), another spanning residues 567-585 (TM-2), a third spanning residues 596-614 (TM-3) and the fourth spanning residues 788-808 (TM-4). Based on this assignment, it is likely that the human

GluR1B receptor structure, in its natural membrane-bound form, consists of a 520 amino acid N-terminal extracellular domain, followed by a hydrophobic region containing four transmembrane domains and an extracellular, 80 amino acid C-terminal domain.

The GluR2B receptor, in precursor form bears a 21 amino acid residue N-terminal signal peptide, and in mature form, consists of 862 amino acids arranged in the sequence illustrated, by single letter code, in Figure 2 (SEQ ID NOS: 3 and 4). With respect to structural domains of the receptor, hydropathy analysis reveals four putative transmembrane domains, one spanning residues 525-544 inclusive (TM-1), another spanning residues 571-589 (TM-2), a third spanning residues 600-618 (TM-3) and the fourth spanning residues 792-812 (TM-4). Based on this assignment, it is likely that the human GluR2B receptor structure, in its natural membrane-bound form, consists of a 524 amino acid N-terminal extracellular domain, followed by a hydrophobic region containing four transmembrane domains and an extracellular, 50 amino acid C-terminal domain.

The GluR3A member of the human GluR family bears a 22 amino acid residue N-terminal signal peptide in precursor form, and is transported to the cell surface in mature form, lacking the signal peptide and consisting of 866 amino acids arranged in the sequence illustrated, by single letter code, in Figure 3 (SEQ ID NOS: 5 and 6). The four putative transmembrane domains of the GluR3A receptor are as follows: one spans residues 527-546 inclusive (TM-1), another spans residues 575-593 (TM-2), a third spans residues 604-622 (TM-3) and the fourth spans residues 796-816 (TM-4). Based on this assignment, it is likely that the human GluR3A receptor structure, in its natural membrane-bound form, consists of a 526 amino acid N-terminal extracellular domain, followed by a hydrophobic region containing four transmembrane domains and an extracellular, 50 amino acid C-terminal domain.

Structurally related variants of the GluR parent receptors identified above also exist. Specifically, a structurally related variant of the human GluR3A receptor, namely the GluR3B receptor, has also been identified. This variant occurs naturally in human brain tissue, and like GluR3A, the GluR3B receptor is 866 amino acids in length, as shown in Figure 4 (SEQ ID NOS: 7 and 8), in its mature, membrane-bound form. The GluR3B receptor initially bears a signal peptide identical to that borne on the GluR3A receptor. Four transmembrane domains are also apparent from the GluR3B sequence, and indicate that these domains lie in the same amino acid regions identified in connection with the GluR3A receptor.

With respect to primary structure, the human GluR3B receptor differs from the GluR3A receptor in a 36 amino acid region separating transmembrane domains TM-3 and TM-4, i.e. residues 748-783. For comparison, the sequences of GluR3A and GluR3B in this region are compared in Figure 5 (SEQ ID NOS: 9 and 10).

Binding assays performed with various ligands, and with membrane preparations derived from mammalian cells engineered genetically to produce the human GluR receptors in membrane-bound form indicate that the human GluR receptors bind selectively to AMPA, relative particularly to kainate and NMDA. This feature, coupled with the medically significant connection between AMPA-type receptors and neurological disorders and disease indicate that the present receptors, as well as AMPA-binding fragments and variants thereof, will serve as valuable tools in the screening and discovery of ligands useful to modulate *in vivo* interactions between such receptors and their natural ligand, glutamate. Thus, a key aspect of the present invention resides in the construction of cells that are engineered genetically to produce a human GluR receptor, to serve as a ready and homogeneous source of receptor for use in *vitro* ligand binding and/or channel activation assays.

For use in the ligand binding assays, it is desirable to construct by application of genetic engineering techniques a host cell, either prokaryotic or eukaryotic, that produces a human GluR receptor as a heterologous and membrane-bound product. According to one embodiment of the invention, the construction of such engineered cells is achieved by introducing into a selected host cell a recombinant DNA construct in which DNA coding for a secretable form of the desired human GluR receptor, i.e., a form bearing its native signal peptide or a functional, heterologous equivalent thereof, is linked operably with expression controlling elements that are functional in the selected host to drive expression of the receptor-encoding DNA, and thus elaborate the desired human GluR receptor protein. Such cells are herein characterized as having the receptor-encoding DNA incorporated "expressibly" therein. The receptor-encoding DNA is referred to as "heterologous" with respect to the particular cellular host if such DNA is not naturally found in the particular host. The particular cell type selected to serve as host for production of the human GluR receptor can be any of several cell types currently available in the art, including both prokaryotic and eukaryotic cells, but should not of course be a cell type that in its natural state elaborates a surface receptor that can bind excitatory amino acids, and so confuse the assay results sought from the engineered cell line. Generally, such problems are avoided by selecting as host a non-neuronal cell type, and can further be avoided using non-human cell lines, as is conventional. It will be appreciated that neuronal- and human-type cells may nevertheless serve as expression hosts, provided that "background" binding to the test ligand is accounted for in the assay results.

According to one embodiment of the present invention, the cell line selected to serve as host for human GluR receptor production is a mammalian cell. Several types of such cell lines are currently available for ge-

netic-engineering work, and these include the chinese hamster ovary (CHO) cells for example of K1 lineage (ATCC CCL 61) including the Pro5 variant (ATCC CRL 1281); the fibroblast-like cells derived from SV40-transformed African Green monkey kidney of the CV-1 lineage (ATCC CCL 70), of the COS-1 lineage (ATCC CRL 1650) and of the COS-7 lineage (ATCC CRL 1651); murine L-cells, murine 3T3 cells (ATCC CRL 1658), murine C127 cells, human embryonic kidney cells of the 293 lineage (ATCC CRL 1573), human carcinoma cells including those of the HeLa lineage (ATCC CCL 2), and neuroblastoma cells of the lines IMR-32 (ATCC CCL 127), SK-N-MC (ATCC HTB 10) and SK-N-SH (ATCC HTB 11).

A variety of gene expression systems have been adapted for use with these hosts and are now commercially available, and any one of these systems can be selected to drive expression of human GluR receptor-encoding DNA. These systems, available typically in the form of plasmidic vectors, incorporate expression cassettes the functional components of which include DNA constituting expression controlling sequences, which are host-recognized and enable expression of the receptor-encoding DNA when linked 5' thereof. The systems further incorporate DNA sequences which terminate expression when linked 3' of the receptor-encoding region. Thus, for expression in the selected mammalian cell host, there is generated a recombinant DNA expression construct in which DNA coding for a secretable form of the receptor is linked with expression controlling DNA sequences recognized by the host, and which include a region 5' of the receptor-encoding DNA to drive expression, and a 3' region to terminate expression. The plasmidic vector harboring the recombinant DNA expression construct typically incorporates such other functional components as an origin of replication, usually virally-derived, to permit replication of the plasmid in the expression host and desirably also for plasmid amplification in a bacterial host, such as E.coli. To provide a marker-enabling selection of stably transformed recombinant cells, the vector will also incorporate a gene conferring some survival advantage on the transformants, such as a gene coding for neomycin resistance in which case the transformants are plated in medium supplemented with neomycin.

Included among the various recombinant DNA expression systems that can be used to achieve mammalian cell expression of the receptor-encoding DNA are those that exploit promoters of viruses that infect mammalian cells, such as the promoter from the cytomegalovirus (CMV), the Rous sarcoma virus (RSV), simian virus (SV40), murine mammary tumor virus (MMTV) and others. Also useful to drive expression are promoters such as the LTR of retroviruses, insect cell promoters such as those regulated by temperature, and isolated from Drosophila, as well as mammalian gene promoters such as those regulated by heavy metals i.e.the metalothionein gene promoter, and other steroid-inducible promoters.

For incorporation into the recombinant DNA expression vector, DNA coding for a selected human GluR receptor, e.g. one of the human GluR1B, GluR2B or GluR3A receptors, or an AMPA-binding fragment or variant thereof, e.g. GluR3B, can be obtained by applying selected techniques of gene isolation or gene synthesis. As described in more detail in the examples herein, human GluR receptors are encoded within the genome of human brain tissue, and can therefore be obtained from human DNA libraries by careful application of conventional gene isolation and cloning techniques. This typically will entail extraction of total messenger RNA from a fresh source of human brain tissue, preferably cerebellum or hippocampus tissue, followed by conversion of message to cDNA and formation of a library in for example a bacterial plasmid, more typically a bacteriophage. Such bacteriophage harboring fragments of the human DNA are typically grown by plating on a lawn of susceptible E. coli bacteria, such that individual phage plaques or colonies can be isolated. The DNA carried by the phage colony is then typically immobilized on a nitrocellulose or nylon-based hybridization membrane, and then hybridized, under carefully controlled conditions, to a radioactively (or otherwise) labelled oligonucleotide probe of appropriate sequence to identify the particular phage colony carrying receptor-encoding DNA or fragment thereof. It will be understood, for example, that selective hybridization, i.e. hybridization of a DNA sequence that is completely complementary to the probe, will be conducted under stringent hybridization conditions. Typically, the gene or a portion thereof so identified is subcloned into a plasmidic vector for nucleic acid sequence analysis.

In specific embodiments of the invention, the GluR1B receptor is encoded by the DNA sequence illustrated in Figure 1 (SEQ ID NO: 1), the GluR2B receptor is encoded by the DNA sequence illustrated in Figure 2 (SEQ ID NO: 3) and the GluR3A and GluR3B receptors are encoded by the DNA sequences illustrated respectively in Figures 3 (SEQ ID NO: 5) and 4 (SEQ ID NO: 7). Alternatively, codons within the illustrated DNA sequences coding for the GluR receptors may be replaced by synonymous codon equivalents, such synonymous codon replacements being well-known in the art.

The illustrated DNA sequences constitute cDNA sequences identified in human brain cDNA libraries in the manner exemplified herein. Having herein provided the nucleotide sequence of various members of the human GluR receptor family, however, it will be appreciated that polynucleotides encoding the receptors can be obtained by other routes. Automated techniques of gene synthesis and/or amplification can be performed to generate DNA coding therefor. Because of the length of the human GluR receptor-encoding DNA, application of

automated synthesis may require staged gene construction, in which regions of the gene up to about 300 nucleotides in length are synthesized individually and then ligated in correct succession by overhang complementarity for final assembly. Individually synthesized gene regions can be-amplified prior to assembly, using established polymerase chain reaction (PCR) technology.

By the application of automated gene synthesis techniques, there is provided a means to generate polynucleotides that encode variants of naturally occurring human GluR receptors, i.e. GluR1B, GluR2B, GluR3A and GluR3B. It will be appreciated, for example, that polynucleotides coding for the human GluR receptors herein described can be generated by substituting synonymous codons for those represented in the naturally occurring polynucleotide sequences herein identified. In addition, polynucleotides coding for human GluR receptor variants can be generated which for example incorporate one or more e.g. 1-10, single amino acid substitutions, deletions or additions. Since it will for the most part be desirable to retain the natural ligand binding profile of the receptor for screening purposes, it is desirable to limit amino acid substitutions, for example to the so-called conservative replacements in which amino acids of like charge are substituted, and to limit substitutions to those sites less critical for receptor activity e.g. within about the first 20 N-terminal residues of the mature receptor, and such other regions as are elucidated upon receptor domain mapping.

With appropriate template DNA in hand, the technique of PCR amplification may also be used to directly generate all or part of the final gene. In this case, primers are synthesized which will prime the PCR amplification of the final product, either in one piece, or in several pieces that may be ligated together. This may be via step-wise ligation of blunt ended, amplified DNA fragments, or preferentially via step-wise ligation of fragments containing naturally occurring restriction endonuclease sites. In this application, it is possible to use either cDNA or genomic DNA as the template for the PCR amplification. In the former case, the cDNA template can be obtained from commercially available or self-constructed cDNA libraries of various human brain tissues, including hippocampus and cerebellum.

Once obtained, the receptor-encoding DNA is incorporated for expression into any suitable expression vector, and host cells are transfected therewith using conventional procedures, such as DNA-mediated transformation, electroporation, or particle gun transformation. Expression vectors may be selected to provide transformed cell lines that express the receptor-encoding DNA either transiently or in a stable manner. For transient expression, host cells are typically transformed with an expression vector harboring an origin of replication functional in a mammalian cell. For stable expression, such replication origins are unnecessary, but the vectors will typically harbour a gene coding for a product that confers on the transformants a survival advantage, to enable their selection. Genes coding for such selectable markers include the E. coli *gpt* gene which confers resistance to mycophenolic acid, the *neo* gene from transposon Tn5 which confers resistance to the antibiotic G418 and to neomycin, the *dhfr* sequence from murine cells or E. coli which changes the phenotype of DHFR- cells into DHFR+ cells, and the *tk* gene of herpes simplex virus, which makes TK- cells phenotypically TK+ cells. Both transient expression and stable expression can provide transformed cell lines, and membrane preparations derived therefrom, for use in ligand screening assays.

For use in screening assays, cells transiently expressing the receptor-encoding DNA can be stored frozen for later use, but because the rapid rate of plasmid replication will lead ultimately to cell death, usually in a few days, the transformed cells should be used as soon as possible. Such assays may be performed either with intact cells, or with membrane preparations derived from such cells. The membrane preparations typically provide a more convenient substrate for the ligand binding experiments, and are therefore preferred as binding substrates. To prepare membrane preparations for screening purposes, i.e., ligand binding experiments, frozen intact cells are homogenized while in cold water suspension and a membrane pellet is collected after centrifugation. The pellet is then washed in cold water, and dialyzed to remove endogenous EAA ligands such as glutamate, that would otherwise compete for binding in the assays. The dialyzed membranes may then be used as such, or after storage in lyophilized form, in the ligand binding assays. Alternatively, intact, fresh cells harvested about two days after transient transfection or after about the same period following fresh plating of stably transfected cells, can be used for ligand binding assays by the same methods as used for membrane preparations. When cells are used, the cells must be harvested by more gentle centrifugation so as not to damage them, and all washing must be done in a buffered medium, for example in phosphate-buffered saline, to avoid osmotic shock and rupture of the cells.

The binding of a substance, i.e., a candidate ligand, to a human GluR receptor of the invention is evaluated typically using a predetermined amount of cell-derived membrane (measured for example by protein determination), generally from about 25μg to 100μg. Generally, competitive binding assays will be useful to evaluate the affinity of a test compound relative to AMPA. This competitive binding assay can be performed by incubating the membrane preparation with radiolabelled AMPA, for example [^{3}H]-AMPA, in the presence of unlabelled test compound added at varying concentrations. Following incubation, either displaced or bound radiolabelled AMPA can be recovered and measured, to determine the relative binding affinities of the test compound

and AMPA for the particular receptor used as substrate. In this way, the affinities of various compounds for the AMPA-binding human EAA receptors can be measured. Alternatively, a radiolabelled analogue of glutamate may be employed in place of radiolabelled AMPA, as competing ligand.

As an alternative to using cells that express receptor-encoding DNA, ligand characterization may also be performed using cells for example Xenopus oocytes, that yield functional membrane-bound receptor following introduction by injection either of receptor-encoding messenger RNA into the oocyte cytoplasm, or of receptor-encoding DNA into the oocyte nucleus. To generate the messenger RNA of cytoplasmic delivery, the receptor-encoding DNA is typically subcloned first into a plasmidic vector adjacent a suitable promoter region, such as the T3 or T7 bacteriophage promoters, to enable transcription into RNA message. RNA is then transcribed from the inserted gene *in vitro,* collected and then injected into Xenopus oocytes. Following the injection of nL volumes of an RNA solution, the oocytes are left to incubate for up to several days, and are then tested for the ability to respond to a particular ligand molecule supplied in a bathing solution. Since functional EAA receptors act in part by operating a membrane channel through which ions may selectively pass, the functioning of the receptor in response to a particular ligand molecule in the bathing solution may typically be measured as an electrical current utilizing microelectrodes inserted into the cell, in the established manner.

In addition to using the receptor-encoding DNA to construct cell lines useful for ligand screening, expression of the DNA can, according to another aspect of the invention, be performed to produce AMPA-binding fragments of the receptor in soluble form, for structure investigation, to raise antibodies and for other experimental uses. It is expected that the portion of the human GluR receptor responsible for AMPA-binding resides on the outside of the cell, i.e., is extracellular. It is therefore desirable in the first instance to facilitate the characterization of the receptor-ligand interaction by providing this extracellular ligand-binding domain in quantity and in isolated form, i.e., free from the remainder of the receptor. To accomplish this, the full-length human GluR receptor-encoding DNA may be modified by site-directed mutagenesis, so as to introduce a translational stop codon into the extracellular N-terminal region, immediately before the sequence encoding the first transmembrane domain (TM1), i.e., before residue 521 of GluR1B, before residue 525 in GluR2B, or before residue 527 of GluR3A and GluR3B. Since there will no longer be produced any transmembrane domain(s) to "anchor" the receptor into the membrane, expression of the modified gene will result in the secretion, in soluble form, of only the extracellular ligand-binding domain. Standard ligand-binding assays may then be performed to ascertain the degree of binding of a candidate compound to the extracellular domain so produced. It may of course be necessary, using site-directed mutagenesis, to produce several different versions of the extracellular regions, in order to optimize the degree of ligand binding to the isolated domains.

For use in ligand binding assays according to the present invention, AMPA-binding fragments of the receptor will first be anchored to a solid support using any one of various techniques. In one method, the C-terminal end of the receptor peptide fragment may be coupled to a derivatized, insoluble polymeric support, for example, cross-linked polystyrene or polyamide resin. Once anchored to the solid support, the frament is useful to screen candidate ligands for receptor binding affinity. For this purpose, competition-type ligand-binding assays, as described above using full-length receptor, are commonly used. Fragments secured to a solid support are bound with a natural ligand, i.e. AMPA, in the presence of a candidate ligand. One of AMPA or candidate ligand is labelled, for example radioactively, and following a suitable incubation period, the degree of AMPA displacement is determined by measuring the amount of bound or unbound label.

Alternatively, it may be desirable to produce an extracellular domain of the receptor which is not derived from the amino-terminus of the mature protein, but rather from the carboxy-terminus instead, for example domains immediately following the fourth transmembrane domain (TM4), i.e., residing between amino acid residues 809-888 of GluR1B, residues 813-862 of GluR2B, or residues 817-866 of GluR3A or GluR3B. In this case, site-directed mutagenesis and/or PCR-based amplification techniques may readily be used to provide a defined fragment of the gene encoding the receptor domain of interest. Such a DNA sequence may be used to direct the expression of the desired receptor fragment, either intracellularly, or in secreted fashion, provided that the DNA encoding the gene fragment is inserted adjacent to a translation start codon provided by the expression vector, and that the required translation reading frame is carefully conserved.

It will be appreciated that the production of such AMPA-binding fragments of a GluR receptor may be accomplished in a variety of host cells. Mammalian cells such as CHO cells may be used for this purpose, the expression typically being driven by an expression promoter capable of high-level expression, for example the CMV (cytomegalovirus) promoter. Alternately, non-mammalian cells, such as insect Sf9 (Spodoptera frugiperda) cells may be used, with the expression typically being driven by expression promoters of the baculovirus, for example the strong, late polyhedrin protein promoter. Filamentous fungal expression systems may also be used to secrete large quantities of such extracellular domains of the EAA receptor. Aspergillus nidulans, for example, with the expression being driven by the alcA promoter, would constitute such an acceptable system. In addition to such expression hosts, it will be further appreciated that any prokaryotic or other eukaryotic ex-

pression system capable of expressing heterologous genes or gene fragments, whether intracellularly or extracellularly would be similarly acceptable.

For use particularly in detecting the presence and/or location of a human GluR receptor, for example in brain tissue, the present invention also provides, in another of its aspects, labelled antibody to a human GluR receptor. To raise such antibodies, there may be used as immunogen either the intact, soluble receptor or an immunogenic fragment thereof i.e. a fragment capable of eliciting an immune response, produced in a microbial or mammalian cell host as described above or by standard peptide synthesis techniques. Regions of human GluR receptor particularly suitable for use as immunogenic fragments include those corresponding in sequence to an extracellular region of the receptor, or a portion of the extracellular region. For example, peptides consisting of residues 1-526 of the GluR3A receptor or a fragment thereof comprising at least about 10 residues, including particularly fragments containing residues 178-193 or 479-522; and peptides corresponding to the region between transmembrane domains TM-2 and TM-3 of the GluR3A receptor, such as a peptide consisting of residues 594-603. Peptides consisting of the C-terminal domain (residues 817-866 of the GluR3A receptor), or fragment thereof, may also be used for the raising of antibodies.

The raising of antibodies to the selected human GluR receptor or immunogenic fragment can be achieved, for polyclonal antibody production, using immunization protocols of conventional design, and any of a variety of mammalian hosts, such as sheep, goats and rabbits. Alternatively, for monoclonal antibody production, immunocytes such as splenocytes can be recovered from the immunized animal and fused, using hybridoma technology, to a myeloma cells. The fusion products are then screened by culturing in a selection medium, and cells producing antibody are recovered for continuous growth, and antibody recovery. Recovered antibody can then be coupled covalently to a detectable label, such as a radiolabel, enzyme label, luminescent label or the like, using linker technology established for this purpose.

In detectably labelled form, e.g. radiolabelled form, DNA or RNA coding for a human GluR receptor, and selected regions thereof, may also be used, in accordance with another aspect of the present invention, as hybridization probes for example to identify sequence-related genes resident in the human or other mammalian genomes (or cDNA libraries) or to locate the human GluR-encoding DNA in a specimen, such as brain tissue. This can be done using either the intact coding region, or a fragment thereof having radiolabelled e.g. ^{32}P, nucleotides incorporated therein. To identify the human GluR-encoding DNA in a specimen, it is desirable to use either the full length cDNA coding therefor, or a fragment which is unique thereto. With reference to Figures 1-4 (SEQ ID NOS: 1-8), such nucleotide fragments include those comprising at least about 17 nucleic acids, and otherwise corresponding in sequence to a region coding for an extracellular N-terminal or C-terminal region of the receptor, or representing a 5'-untranslated or 3'-untranslated region thereof. Such oligonucleotide sequences, and the intact gene itself, may also be used of course to clone human GluR-related human genes, particularly cDNA equivalents thereof, by standard hybridization techniques.

Embodiments of the present invention are described in detail in the following specific examples which are not to be construed as limiting:

Example 1 - Isolation of DNA coding for the human GluR3A receptor

The particular strategy used to clone the human GluR3A receptor is depicted schematically in Figure 6, and described in greater detail below.

cDNA coding for the human GluR3A receptor was identified by probing human hippocampal cDNA that was obtained as an EcoRI-based lambda phage library (lambda ZAP) from Stratagene Cloning Systems (La Jolla, California, U.S.A.). The cDNA library was probed initially with a 1.1kb EcoRI/EcoRI DNA fragment constituting the 3' region of a kainate-binding human EAA receptor, designated humEAAla. This particular kainate-binding receptor is described in EP-A-0 529 994 incorporated herein by reference. DNA coding for the human EAA1a receptor, and from which the 1.1kb probe may be recovered, was deposited under terms of the Budapest Treaty, with the American Type Culture Collection in Rockville, Maryland U.S.A. on August 21, 1991 under accession number ATCC 75063.

Hybridizations using the probe were carried out at 30C overnight, and filters were washed with 2xSSC containing 0.5% SDS at 25C for 5 minutes, followed by a 15 minute wash at 50C with 2xSSC containing 0.5% SDS. The final wash was with 1xSSC containing 0.5% SDS at 50C for 15 minutes. Filters were exposed to X-ray film (Kodak) overnight. Of 10^6 clones screened under the following hybridization conditions (6xSSC, 50% formamide, 5% Denhardt's solution, 0.5% SDS, 100ug/ml denatured salmon sperm DNA), only two hippocampal cDNA library inserts were identified, one about 1.6kb and designated RKCH521 and another about 2.2kb and designated RKCH221 (Fig.6). For sequencing, the '521 and the '221 phages were plaque purified, then excised as phagemids according to the supplier's specifications, to generate insert-carrying Bluescript-SK variants of the phagemid vector. Sequencing of the '221 clone across its entire sequence revealed a putative ATG

initiation codon together with about 78 bases of 5'non-coding region and about 2.1 kb of coding region. Sequencing across the '521 insert revealed a significant region of overlap with the '221 insert, and provided some additional 3' sequence, although no termination codon was located.

There being no termination codon apparent in the '521 sequence, a 3' region of the gene was sought. For this purpose, there was first synthesized an oligonucleotide probe capable of annealing to the 3' region of the rat GluR3 receptor sequence reported by Keinanen et al, supra. The specific sequence of the 32-P-labelled probe is provided below (SEQ ID NO: 13):

5' - ACACTCAGAATTACGCTACATACAGAGAAGGCTACAACGT - 3'

The same hippocampal cDNA library was then re-screened using the rat-based probe and under the following hybridization conditions; 6xSSC, 25% formamide, 5% Dernhardt's solution, 0.5% SDS, 100ug/ml denatured salmon sperm DNA, 42C. This revealed a 1.2kb insert, designated RKCSHG132. Sequencing of the entire insert revealed 5' overlap with the 3'end of the previously isolated '521 insert, and also revealed a termination codon as well as about 15 bases of 3'non-translated sequence.

To provide the entire coding region in an intact clone, the strategy shown in Figure 6 was employed, to generate the phagemid pBS/HumGluR3A which carries the hGluR3A-encoding DNA as a 2.8kb EcoRI/EcoRI insert in a 3.0kb Bluescript-SK phagemid background. The entire sequence of the EcoRI/EcoRI insert is provided in Figure 3 (SEQ ID NOS: 5 and 6).

The 5.8kb phagemid pBS/humGluR3A was deposited, under the terms of the Budapest Treaty, with the American Type Culture Collection in Rockville, Maryland USA on March 19, 1992, and has been assigned accession number ATCC 75218.

Example 2 - Isolation of DNA coding for human GluR3B receptor

A human fetal brain cDNA library was also screened in the search for human GluR receptors. This particular library was obtained as an EcoRI-based lambda gt10 library from Strategene Cloning Systems (La Jolla, California, U.S.A.). The library was first screened using as hybridization probe an oligonucleotide capable of hybridizing to a 3' region of the reported rat GluR3 gene sequence. Screening using hybridization conditions as noted above (6xSSC, 25% formamide, 42C, etc.) revealed one insert about 2.3kb in size, designated RKCSFG34. After excision to release Bluescript-SK phagemids carrying the insert, sequencing revealed substantial sequence identity between the '34 insert and the 3'end of the earlier isolated GluR3A clone, and suggested that the 5'end of the gene encoded on partially on the '34 insert was missing. To provide an assembled gene, a 5' region was excised from the GluR3A insert and used to generate the 5'end of the '34 insert, at an internal HindIII site. This was achieved as depicted schematically in Figure 7. The resulting intact clone was designated human GluR3B.

Sequence comparison between the GluR3A clone of Example 1 and the GluR3B clone of this Example revealed only a short region of dissimilarity which is illustrated, in terms of amino acid sequence, in Figure 5 (SEQ ID NOS: 9 and 10).

The 6.1kb phagemid pBS/humGluR3B was deposited, under the terms of the Budapest Treaty, with the American Type Culture Collection in Rockville, Maryland USA on March 19, 1992, and has been assigned accession number ATCC 75219.

Example 3 - Isolation of DNA coding for the human GluR1B receptor

cDNA coding for the human GluR1B receptor was identified by probing human fetal brain cDNA that was obtained as an EcoRI-based lambda phage library (lambda ZAP) from Stratagene Cloning Systems (La Jolla, California, U.S.A.). The cDNA library was screened using an oligonucleotide probe capable of annealing to the 5' region of the rat GluR1 receptor sequence reported by Hollmann et al, supra. The specific sequence of the 32-P-labelled probe is provided below (SEQ ID NO: 14):

5' - CCAGATCGATATTGTGAACATCAGCGACACGTTTGAGATG - 3'

The fetal brain cDNA library was screened under the following hybridization conditions; 6xSSC, 25% formamide, 5% Dernhardt's solution, 0.5% SDS, 100ug/ml denatured salmon sperm DNA, 42C. Filters were washed with 2xSSC containing 0.5% SDS at 25C for 5 minutes, followed by a 15 minute wash at 50C with 2xSSC containing 0.5% SDS. The final wash was with 1xSSC containing 0.5% SDS at 50C for 15 minutes. Filters were exposed to X-ray film (Kodak) overnight. Of 10^6 clones screened, only one cDNA insert, of about 3.2kb, was

identified, and designated RKCSFG91. For sequencing, the '91 phage was plaque purified, then excised as a phagemid according to the supplier's specifications, to generate an insert-carrying Bluescript-SK variant of the phagemid vector. Sequencing of the '91 clone across its entire sequence revealed a putative ATG initiation codon together with about 61 bases of 5'non-coding region and 2,718 bases of coding region. Also revealed was a termination codon, as well as about 438 bases of 3' non-translated sequence. The entire sequence of the EcoRI/EcoRI insert is provided in Figure 1 (SEQ ID NOS: 1 and 2).

A 6.2kb phagemid designated pBS/humGluR1B, carrying the receptor-encoding DNA as a 3.2kb EcoRI/EcoRI insert in a 3.0kb Bluescript-SK phagemid background, was deposited, under the terms of the Budapest Treaty, with the American Type Culture Collection in Rockville, Maryland USA on May 28, 1992, and has been assigned accession number ATCC 75246.

Example 4 - Isolation of DNA coding for the human GluR2B receptor

The particular strategy used to clone the human Glu2B receptor is depicted schematically in Figure 10, and described in greater detail below.

cDNA coding for the human GluR2B receptor was identified by probing human hippocampal cDNA that was obtained as an EcoRI-based lambda phage library (lambda ZAP) from Stratagene Cloning Systems (La Jolla, California, U.S.A.). The cDNA library was screened using an oligonucleotide probe capable of annealing to the 3' region of the rat GluR2 receptor sequence reported by Keinanen et al, supra. The specific sequence of the 32-P-labelled probe is provided below (SEQ ID NO: 15):

5'-GTGAATGTGGAGCCAAGGACTCGGGAAGTAAG-3'

The hippocampal cDNA library was screened under the following hybridization conditions; 6xSSC, 25% formamide, 5% Dernhardt's solution, 0.5% SDS, 100ug/ml denatured salmon sperm DNA, 42C. Filters were washed with 2xSSC containing 0.5% SDS at 25C for 5 minutes, followed by a 15 minute wash at 50C with 2xSSC containing 0.5% SDS. The final wash was with 1xSSC containing 0.5% SDS at 50C for 15 minutes. Filters were exposed to X-ray film (Kodak) overnight. Of 10^6 clones screened, only two cDNA inserts were identified, one about 2.7kb and designated RKCSHG84 and another about 2.9kb and designated RKCSHG41 (Fig.10). For sequencing, the '84 and the '41 phages were plaque purified, then excised as phagemids according to the supplier's specifications, to generate insert-carrying Bluescript-SK variants of the phagemid vector. Sequencing of the '84 clone across its entire sequence revealed a putative ATG initiation codon together with about 314 bases of 5'non-coding region and about 2.4 kb of coding region. Sequencing across the '41 insert revealed a significant region of overlap with the '84 insert, and also revealed a termination codon not found in the '84 insert as well as about 441 bases of 3' non-translated sequence.

To provide the entire coding region in an intact clone, the strategy shown in Figure 10 was employed, to generate the phagemid pBS/HumGluR2B which carries the hGluR2B-encoding DNA as a 3.4kb EcoRI/PstI insert in a 3.Okb Bluescript-SK phagemid background. The entire sequence of the EcoRI/PstI insert is provided in Figure 2 (SEQ ID NOS: 3 and 4).

The 6.4kb phagemid pBS/humGluR2B was deposited, under the terms of the Budapest Treaty, with the American Type Culture Collection in Rockville, Maryland USA on March 19, 1992, and has been assigned accession number ATCC 75217.

Example 5 - Construction of genetically engineered cells producing human GluR3A receptors

The strategy depicted in Figure 8 was employed to facilitate incorporation of the GluR3A receptor-encoding cDNA into an expression vector.

For transient expression in mammalian cells, cDNA coding for the human GluR3A receptor was incorporated into the mammalian expression vector pcDNAI, which is available commercially from Invitrogen Corporation (San Diego, California, USA; catalogue number V490-20). This is a multifunctional 4.2kb plasmid vector designed for cDNA expression in eukaryotic systems, and cDNA analysis in prokaryotes. Incorporated on the vector are the CMV promoter and enhancer, splice segment and polyadenylation signal, an SV40 and Polyoma virus origin of replication, and M13 origin to rescue single strand DNA for sequencing and mutagenesis, Sp6 and T7 RNA promoters for the production of sense and anti-sense RNA transcripts and a Col E1-like high copy plasmid origin. A polylinker is located appropriately downstream of the CMV promoter (and 3' of the T7 promoter).

To facilitate incorporation of the GluR3A receptor-encoding cDNA into an expression vector, a NotI site was introduced onto the 5' flank of the Bluescript-SK cDNA insert, and the cDNA insert was then released from

pBS/humGluR3A as a 2.8 kb NotI/NotI fragment, which was then incorporated at the NotI site in the pcDNAI polylinker. Sequencing across the NotI junction was performed, to confirm proper insert orientation in pcDNAI. The resulting plasmid, designated pcDNAI/humGluR3A, was then introduced for transient expression into a selected mammalian cell host, in this case the monkey-derived, fibroblast like cells of the COS-1 lineage (available from the American Type Culture Collection, Rockville, Maryland as ATCC CRL 1650).

For transient expression of the GluR3A-encoding DNA, COS-1 cells were transfected with approximately 8ug DNA (as pcDNA1/humGluR3A) per 10^6 COS cells, by DEAE-mediated DNA transfection and treated with chloroquine according to the procedures described by Maniatis et al, supra. Briefly, COS-1 cells were plated at a density of 5 x 10^6 cells/dish and then grown for 24 hours in FBS-supplemented DMEM/F12 medium. Medium was then removed and cells were washed in PBS and then in medium. There was then applied on the cells 10ml of a transfection solution containing DEAE dextran (0.4mg/ml), 100uM chloroquine, 10% NuSerum, DNA (0.4mg/ml) in DMEM/F12 medium. After incubation for 3 hours at 37C, cells were washed in PBS and medium as just described and then shocked for 1 minute with 10% DMSO in DMEM/F12 medium. Cells were allowed to grow for 2-3 days in 10% FBS-supplemented medium, and at the end of incubation dishes were placed on ice, washed with ice cold PBS and then removed by scraping. Cells were then harvested by centrifugation at 1000 rpm for 10 minutes and the cellular pellet was frozen in liquid nitrogen, for subsequent use in ligand binding assays. Northern blot analysis of a thawed aliquot of frozen cells confirmed expression of receptor-encoding cDNA in cells under storage.

In a like manner, stably transfected cell lines can also prepared using two different cell types as host: CHO K1 and CHO Pro5. To construct these cell lines, cDNA coding for human GluR3A was incorporated into the mammalian expression vector pRC/CMV (Invitrogen), which enables stable expression. Insertion at this site placed the cDNA under the expression control of the cytomegalovirus promoter and upstream of the polyadenylation site and terminator of the bovine growth hormone gene, and into a vector background comprising the neomycin resistance gene (driven by the SV40 early promoter) as selectable marker.

To introduce plasmids constructed as described above, the host CHO cells are first seeded at a density of 5 x 10^5 in 10% FBS-supplemented MEM medium. After growth for 24 hours, fresh medium are added to the plates and three hours later, the cells are transfected using the calcium phosphate-DNA co-precipitation procedure (Maniatis et al, supra). Briefly, 3ug of DNA is mixed and incubated with buffered calcium solution for 10 minutes at room temperature. An equal volume of buffered phosphate solution is added and the suspension is incubated for 15 minutes at room temperature. Next, the incubated suspension is applied to the cells for 4 hours, removed and cells were shocked with medium containing 15% glycerol. Three minutes later, cells are washed with medium and incubated for 24 hours at normal growth conditions. Cells resistant to neomycin are selected in 10% FBS-supplemented alpha-MEM medium containing G418 (1mg/ml). Individual colonies of G418-resistant cells are isolated about 2-3 weeks later, clonally selected and then propogated for assay purposes.

Example 6 - Construction of genetically engineered cells producing human GluR1B receptors

The strategy depicted in Figure 9 was employed to facilitate incorporation of the GluR1B receptor-encoding cDNA into an expression vector. Particularly, a NotI site was introduced onto the 3' flank of the Bluescript-SK cDNA insert, and the cDNA insert was then released from pBS/humGluR1B as a 3.2kb NotI/NotI fragment, which was then incorporated at the NotI site in the pcDNAI polylinker. Sequencing across the junctions was performed, to confirm proper insert orientation in pcDNA1. The resulting plasmid, designated pcDNA1/ humGluR1B, was then introduced for transient expression into monkey-derived, fibroblast like cells of the COS-1 lineage as described above.

For transient expression of the GluR1B-encoding DNA, COS-1 cells were transfected with approximately 8ug DNA (as pcDNA1/humGluR1B) per 10^6 COS cells using the method described in Example 5.

Example 7 - Construction of genetically engineered cells producing human GluR2B receptors

The strategy depicted in Figure 11 was employed to facilitate incorporation of the GluR2B receptor-encoding cDNA into an expression vector. Particularly, a NotI site was introduced onto the 5' flank of the Bluescript-SK cDNA insert, and the cDNA insert was then released from pBS/humGluR2B as a 3.4kb HindIII/NotI fragment, which was then incorporated at the HindIII/NotI sites in the pcDNAI polylinker. Sequencing across the junctions was performed, to confirm proper insert orientation in pcDNA1. The resulting plasmid, designated pcDNA1/humGluR2B, was then introduced for transient expression into a selected mammalian cell host, in this case the monkey-derived, fibroblast like cells of the COS-1 lineage (available from the American Type Culture Collection, Rockville, Maryland as ATCC CRL 1650).

For transient expression of the GluR2B-encoding DNA, COS-1 cells were transfected with approximately 8ug DNA (as pcDNA1/humGluR2B) per 10^6 COS cells as set out in Example 5.

Example 8 - Ligand binding assays

Transfected cells in the frozen state were resuspended in ice-cold distilled water using a hand homogenizer, sonicated for 5 seconds, and then centrifuged for 20 minutes at 50,000g. The supernatant was discarded and the membrane pellet stored frozen at -70C.

COS cell membrane pellets were suspended in ice cold 50mM Tris-HCl (pH 7.55, 5C) and centrifuged again at 50,000g for 10 minutes in order to remove endogenous glutamate that would compete for binding. Pellets were resuspended in ice cold 50mM Tris-HCl (pH 7.55) buffer and the resultant membrane preparation was used as tissue source for binding experiments described below. Proteins were determined using the Pierce Reagent with BSA as standard.

Binding assays were then performed, using an amount of COS-derived membrane equivalent to from 25-100ug as judged by protein determination and selected radiolabelled ligand. In particular, for AMPA-binding assays, incubation mixtures consisted of 25-100ug tissue protein and D,L-alpha-[5-methyl-^{3}H]amino-3-hydroxy-5-methylisoxazole-4-propionic acid (^{3}H-AMPA, 27.6Ci/mmole, 10nM final) with 0.1M KSCN and 2.5mM $CaCl_2$ in the 1ml final volume. Non-specific binding was determined in the presence of 1mM L-glutamate. Samples were incubated on ice for 60 minutes in plastic minivials, and bound and free ligand was separated by centrifugation for 30 minutes at 50,000g. Pellets were washed twice in 6ml of the cold incubation buffer, then 5ml of Beckman Ready-Protein Plus scintillation cocktail was added, for counting.

For kainate-binding assays, incubation mixtures consisted of 25-100ug tissue protein and [vinylidene-^{3}H] kainic acid (58Ci/mmole, 5nM final) in the cold incubation buffer, 1ml final volume. Non-specific binding was determined in the presence of 1mM L-glutamate. Samples were incubated as for the AMPA-binding assays, and bound and free ligand were separated by rapid filtration using a Brandel cell harvester and GF/B filters pre-soaked in ice-cold 0.3% polyethyleneimine. Filters were washed twice in 6ml of the cold incubation buffer, then placed in scintillation vials with 5ml of Beckman Ready-Protein Plus scintillation cocktail for counting.

Assays performed in this manner, using membrane preparations derived from the human GluR3A receptor-producing COS cells, revealed specific binding of 25-30 fmole/mg protein at 10nM [^{3}H]-AMPA (Figure 14); using membrane preparations derived from the human GluR1B receptor-producing COS cells, specific binding of about 100-150 fmole/mg protein at 10nM [^{3}H]-AMPA was revealed (Figure 12); and using membrane preparations derived from the human GluR2B receptor-producing COS cells, specific binding of 750-850 fmol/mg protein at 10nM [^{3}H]-AMPA was revealed (Figure 13). Mock transfected cells exhibited no specific binding of any of the ligands tested.

Scatchard analysis indicated that the recombinantly expressed human GluR1B and GluR2B receptors each contain a single class of [^{3}H]-labelled AMPA binding sites with a dissociation constants (Kd) of 46 nM (Figure 15) and about 36.3 ± 7.4 nM (Figure 16), respectively. Further, the maximum AMPA-binding of the GluR1B and GluR2B receptors has been found to be 847 and 816 ± 302 fmol/mg protein, respectively.

[^{3}H]-AMPA displacement assays have also been performed for the GluR2B receptors in COS cells to determine the relative binding affinity of selected ligands. These results, as illustrated in Figure 17, indicate the rank order of potency of the ligands in displacing ^{3}H-AMPA binding to the GluR2B receptor to be as follows:

quisqualate = AMPA > DNQX > CNQX > glutamate > domoate > kainate

These results demonstrate clearly that the human GluR receptors bind AMPA with specificity. This activity, coupled with the fact that there is little or no demonstrable binding of either kainate or NMDA, clearly assigns the human GluR receptors to be of the AMPA type of EAA receptor. Furthermore, this binding profile indicates that the receptor is binding in an authentic manner, and can therefore reliably predict the ligand binding "signature" of its non-recombinant counterpart from the human brain. These features make the recombinant receptor especially useful for selecting and characterizing ligand compounds which bind to the receptor, and/or for selecting and characterizing compounds which may act by displacing other ligands from the receptor. The isolation of the GluR receptor genes in substantially pure form, capable of being expressed as a single, homogeneous receptor species, therefore frees the ligand binding assay from the lack of precision introduced when complex, heterogeneous receptor preparations from human and other mammalian brains are used to attempt such characterizations.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:
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(ii) TITLE OF INVENTION: AMPA-BINDING HUMAN GLUTAMATE RECEPTORS

(iii) NUMBER OF SEQUENCES: 15

(iv) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

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(A) APPLICATION NUMBER: Unknown

(vi) PRIOR APPLICATION DATA:
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(vi) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: US 07/896,611
(B) FILING DATE: 10-JUN-1992

(vi) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: US 07/896,612
(B) FILING DATE: 10-JUN-1992

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 3220 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 62..2782

(ix) FEATURE:
(A) NAME/KEY: sig_peptide
(B) LOCATION: 62..115

(ix) FEATURE:
(A) NAME/KEY: mat_peptide
(B) LOCATION: 116..2782

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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  Met Gln His Ile Phe Ala Phe Phe Cys Thr Gly Phe Leu Gly Ala
  -18         -15                 -10                 -5

GTA GTA GGT GCC AAT TTC CCC AAC AAT ATC CAG ATC GGG GGA TTA TTT         154
Val Val Gly Ala Asn Phe Pro Asn Asn Ile Gln Ile Gly Gly Leu Phe
            1               5                  10

CCA AAC CAG CAG TCA CAG GAA CAT GCT GCT TTT AGA TTT GCT TTG TCG         202
Pro Asn Gln Gln Ser Gln Glu His Ala Ala Phe Arg Phe Ala Leu Ser
    15                  20                  25

CAA CTC ACA GAG CCC CCG AAG CTG CTC CCC CAG ATT GAT ATT GTG AAC         250
Gln Leu Thr Glu Pro Pro Lys Leu Leu Pro Gln Ile Asp Ile Val Asn
30                  35                  40                  45

ATC AGC GAC ACG TTT GAG ATG ACC TAT AGA TTC TGT TCC CAG TTC TCC         298
Ile Ser Asp Thr Phe Glu Met Thr Tyr Arg Phe Cys Ser Gln Phe Ser
                50                  55                  60

AAA GGA GTC TAT GCC ATC TTT GGG TTT TAT GAA CGT AGG ACT GTC AAC         346
Lys Gly Val Tyr Ala Ile Phe Gly Phe Tyr Glu Arg Arg Thr Val Asn
            65                  70                  75

ATG CTG ACC TCC TTT TGT GGG GCC CTC CAC GTC TGC TTC ATT ACG CCG         394
Met Leu Thr Ser Phe Cys Gly Ala Leu His Val Cys Phe Ile Thr Pro
        80                  85                  90

AGC TTT CCC GTT GAT ACA TCC AAT CAG TTT GTC CTT CAG CTG CGC CCT         442
Ser Phe Pro Val Asp Thr Ser Asn Gln Phe Val Leu Gln Leu Arg Pro
    95                  100                 105

GAA CTG CAG GAT GCC CTC ATC AGC ATC ATT GAC CAT TAC AAG TGG CAG         490
Glu Leu Gln Asp Ala Leu Ile Ser Ile Ile Asp His Tyr Lys Trp Gln
110                 115                 120                 125

AAA TTT GTC TAC ATT TAT GAT GCC GAC CGG GGC TTA TCC GTC CTG CAG         538
Lys Phe Val Tyr Ile Tyr Asp Ala Asp Arg Gly Leu Ser Val Leu Gln
                130                 135                 140

AAA GTC CTG GAT ACA GCT GCT GAG AAG AAC TGG CAG GTG ACA GCA GTC         586
Lys Val Leu Asp Thr Ala Ala Glu Lys Asn Trp Gln Val Thr Ala Val
            145                 150                 155

AAC ATT TTG ACA ACC ACA GAG GAG GGA TAC CGG ATG CTC TTT CAG GAC         634
Asn Ile Leu Thr Thr Thr Glu Glu Gly Tyr Arg Met Leu Phe Gln Asp
        160                 165                 170

CTG GAG AAG AAA AAG GAG CGG CTG GTG GTG GTG GAC TGT GAA TCA GAA         682
Leu Glu Lys Lys Lys Glu Arg Leu Val Val Val Asp Cys Glu Ser Glu
    175                 180                 185

CGC CTC AAT GCT ATC TTG GGC CAG ATT ATA AAG CTA GAG AAG AAT GGC         730
Arg Leu Asn Ala Ile Leu Gly Gln Ile Ile Lys Leu Glu Lys Asn Gly
190                 195                 200                 205

ATC GGC TAC CAC TAC ATT CTT GCA AAT CTG GGC TTC ATG GAC ATT GAC         778
Ile Gly Tyr His Tyr Ile Leu Ala Asn Leu Gly Phe Met Asp Ile Asp
                210                 215                 220

TTA AAC AAA TTC AAG GAG AGT GGC GCC AAT GTG ACA GGT TTC CAG CTG         826
Leu Asn Lys Phe Lys Glu Ser Gly Ala Asn Val Thr Gly Phe Gln Leu
            225                 230                 235
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GTG AAC TAC ACA GAC ACT ATT CCG GCC AAG ATC ATG CAG CAG TGG AAG      874
Val Asn Tyr Thr Asp Thr Ile Pro Ala Lys Ile Met Gln Gln Trp Lys
        240                 245                 250

AAT AGT GAT GCT CGA GAC CAC ACA CGG GTG GAC TGG AAG AGA CCC AAG      922
Asn Ser Asp Ala Arg Asp His Thr Arg Val Asp Trp Lys Arg Pro Lys
    255                 260                 265

TAC ACC TCT GCG CTC ACC TAC GAT GGG GTG AAG GTG ATG GCT GAG GCT      970
Tyr Thr Ser Ala Leu Thr Tyr Asp Gly Val Lys Val Met Ala Glu Ala
270                 275                 280                 285

TTC CAG AGC CTG CGG AGG CAG AGA ATT GAT ATA TCT CGC CGG GGG AAT     1018
Phe Gln Ser Leu Arg Arg Gln Arg Ile Asp Ile Ser Arg Arg Gly Asn
                290                 295                 300

GCT GGG GAT TGT CTG GCT AAC CCA GCT GTT CCC TGG GGC CAA GGG ATC     1066
Ala Gly Asp Cys Leu Ala Asn Pro Ala Val Pro Trp Gly Gln Gly Ile
            305                 310                 315

GAC ATC CAG AGA GCT CTG CAG CAG GTG CGA TTT GAA GGT TTA ACA GGA     1114
Asp Ile Gln Arg Ala Leu Gln Gln Val Arg Phe Glu Gly Leu Thr Gly
        320                 325                 330

AAC GTG CAG TTT AAT GAG AAA GGA CGC CGG ACC AAC TAC ACG CTC CAC     1162
Asn Val Gln Phe Asn Glu Lys Gly Arg Arg Thr Asn Tyr Thr Leu His
    335                 340                 345

GTG ATT GAA ATG AAA CAT GAC GGC ATC CGA AAG ATT GGT TAC TGG AAT     1210
Val Ile Glu Met Lys His Asp Gly Ile Arg Lys Ile Gly Tyr Trp Asn
350                 355                 360                 365

GAA GAT GAT AAG TTT GTC CCT GCA GCC ACC GAT GCC CAA GCT GGG GGC     1258
Glu Asp Asp Lys Phe Val Pro Ala Ala Thr Asp Ala Gln Ala Gly Gly
                370                 375                 380

GAT AAT TCA AGT GTT CAG AAC AGA ACA TAC ATC GTC ACA ACA ATC CTA     1306
Asp Asn Ser Ser Val Gln Asn Arg Thr Tyr Ile Val Thr Thr Ile Leu
            385                 390                 395

GAA GAT CCT TAT GTG ATG CTC AAG AAG AAC GCC AAT CAG TTT GAG GGC     1354
Glu Asp Pro Tyr Val Met Leu Lys Lys Asn Ala Asn Gln Phe Glu Gly
        400                 405                 410

AAT GAC CGT TAC GAG GGC TAC TGT GTA GAG CTG GCG GCA GAG ATT GCC     1402
Asn Asp Arg Tyr Glu Gly Tyr Cys Val Glu Leu Ala Ala Glu Ile Ala
    415                 420                 425

AAG CAC GTG GGC TAC TCC TAC CGT CTG GAG ATT GTC AGT GAT GGA AAA     1450
Lys His Val Gly Tyr Ser Tyr Arg Leu Glu Ile Val Ser Asp Gly Lys
430                 435                 440                 445

TAC GGA GCC CGA GAC CCT GAC ACG AAG GCC TGG AAT GGC ATG GTG GGA     1498
Tyr Gly Ala Arg Asp Pro Asp Thr Lys Ala Trp Asn Gly Met Val Gly
                450                 455                 460

GAG CTG GTC TAT GGA AGA GCA GAT GTG GCT GTG GCT CCC TTA ACT ATC     1546
Glu Leu Val Tyr Gly Arg Ala Asp Val Ala Val Ala Pro Leu Thr Ile
            465                 470                 475

ACT TTG GTC CGG GAA GAA GTT ATA GAT TTC TCC AAA CCA TTT ATG AGT     1594
Thr Leu Val Arg Glu Glu Val Ile Asp Phe Ser Lys Pro Phe Met Ser
        480                 485                 490

TTG GGG ATC TCC ATC ATG ATT AAA AAA CCA CAG AAA TCC AAG CCG GGT     1642
Leu Gly Ile Ser Ile Met Ile Lys Lys Pro Gln Lys Ser Lys Pro Gly
    495                 500                 505
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GTC TTC TCC TTC CTT GAT CCT TTG GCT TAT GAG ATT TGG ATG TGC ATT      1690
Val Phe Ser Phe Leu Asp Pro Leu Ala Tyr Glu Ile Trp Met Cys Ile
510                 515                 520                 525

GTT TTT GCC TAC ATT GGA GTG AGT GTT GTC CTC TTC CTG GTC AGC CGC      1738
Val Phe Ala Tyr Ile Gly Val Ser Val Val Leu Phe Leu Val Ser Arg
                530                 535                 540

TTC AGT CCC TAT GAA TGG CAC AGT GAA GAG TTT GAG GAA GGA CGG GAC      1786
Phe Ser Pro Tyr Glu Trp His Ser Glu Glu Phe Glu Glu Gly Arg Asp
            545                 550                 555

CAG ACA ACC AGT GAC CAG TCC AAT GAG TTT GGG ATA TTC AAC AGT TTG      1834
Gln Thr Thr Ser Asp Gln Ser Asn Glu Phe Gly Ile Phe Asn Ser Leu
        560                 565                 570

TGG TTC TCC CTG GGA GCC TTC ATG CAG CAA GGA TGT GAC ATT TCT CCC      1882
Trp Phe Ser Leu Gly Ala Phe Met Gln Gln Gly Cys Asp Ile Ser Pro
    575                 580                 585

AGG TCC CTG TCT GGT CGC ATC GTT GGT GGC GTC TGG TGG TTC TTC ACC      1930
Arg Ser Leu Ser Gly Arg Ile Val Gly Gly Val Trp Trp Phe Phe Thr
590                 595                 600                 605

TTA ATC ATC ATC TCC TCA TAT ACA GCC AAT CTG GCC GCC TTC CTG ACC      1978
Leu Ile Ile Ile Ser Ser Tyr Thr Ala Asn Leu Ala Ala Phe Leu Thr
                610                 615                 620

GTG GAG AGG ATG GTG TCT CCC ATT GAG AGT GCA GAG GAC CTA GCG AAC      2026
Val Glu Arg Met Val Ser Pro Ile Glu Ser Ala Glu Asp Leu Ala Asn
            625                 630                 635

GAG ACA GAA ATT GCC TAC GGG ACG CTG GAA GCA GGA TCT ACT AAG GAG      2074
Glu Thr Glu Ile Ala Tyr Gly Thr Leu Glu Ala Gly Ser Thr Lys Glu
        640                 645                 650

TTC TTC AGG AGG TCT AAA ATT GCT GTG TTT GAG AAG ATG TGG ACA TAC      2122
Phe Phe Arg Arg Ser Lys Ile Ala Val Phe Glu Lys Met Trp Thr Tyr
    655                 660                 665

ATG AAG TCA GCA GAG CCA TCA GTT TTT GTG CGG ACC ACA GAG GAG GGG      2170
Met Lys Ser Ala Glu Pro Ser Val Phe Val Arg Thr Thr Glu Glu Gly
670                 675                 680                 685

ATG ATT CGA GTG AGG AAA TCC AAA GGC AAA TAT GCC TAC CTC CTG GAG      2218
Met Ile Arg Val Arg Lys Ser Lys Gly Lys Tyr Ala Tyr Leu Leu Glu
                690                 695                 700

TCC ACC ATG AAT GAG TAC ATT GAG CAG CGG AAA CCC TGT GAC ACC ATG      2266
Ser Thr Met Asn Glu Tyr Ile Glu Gln Arg Lys Pro Cys Asp Thr Met
            705                 710                 715

AAG GTG GGA GGT AAC TTG GAT TCC AAA GGC TAT GGC ATT GCA ACA CCC      2314
Lys Val Gly Gly Asn Leu Asp Ser Lys Gly Tyr Gly Ile Ala Thr Pro
        720                 725                 730

AAG GGG TCT GCC CTG AGA GGT CCC GTA AAC CTA GCG GTT TTG AAA CTC      2362
Lys Gly Ser Ala Leu Arg Gly Pro Val Asn Leu Ala Val Leu Lys Leu
    735                 740                 745

AGT GAG CAA GGC GTC TTA GAC AAG CTG AAA AGC AAA TGG TGG TAC GAT      2410
Ser Glu Gln Gly Val Leu Asp Lys Leu Lys Ser Lys Trp Trp Tyr Asp
750                 755                 760                 765

AAA GGG GAA TGT GGA AGC AAG GAC TCC GGA AGT AAG GAC AAG ACA AGC      2458
Lys Gly Glu Cys Gly Ser Lys Asp Ser Gly Ser Lys Asp Lys Thr Ser
                770                 775                 780
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GCT CTG AGC CTC AGC AAT GTG GCA GGC GTG TTC TAC ATC CTG ATC GGA        2506
Ala Leu Ser Leu Ser Asn Val Ala Gly Val Phe Tyr Ile Leu Ile Gly
            785                 790                 795

GGA CTT GGA CTA GCC ATG CTG GTT GCC TTA ATC GAG TTC TGC TAC AAA        2554
Gly Leu Gly Leu Ala Met Leu Val Ala Leu Ile Glu Phe Cys Tyr Lys
        800                 805                 810

TCC CGT AGT GAA TCC AAG CGG ATG AAG GGT TTT TGT TTG ATC CCA CAG        2602
Ser Arg Ser Glu Ser Lys Arg Met Lys Gly Phe Cys Leu Ile Pro Gln
    815                 820                 825

CAA TCC ATC AAC GAA GCC ATA CGG ACA TCG ACC CTC CCC CGC AAC AGC        2650
Gln Ser Ile Asn Glu Ala Ile Arg Thr Ser Thr Leu Pro Arg Asn Ser
830                 835                 840                 845

GGG GCA GGA GCC AGC AGC GGC GGC AGT GGA GAG AAT GGT CGG GTG GTC        2698
Gly Ala Gly Ala Ser Ser Gly Gly Ser Gly Glu Asn Gly Arg Val Val
                850                 855                 860

AGC CAT GAC TTC CCC AAG TCC ATG CAA TCG ATT CCT TGC ATG AGC CAC        2746
Ser His Asp Phe Pro Lys Ser Met Gln Ser Ile Pro Cys Met Ser His
            865                 870                 875

AGT TCA GGG ATG CCC TTG GGA GCC ACG GGA TTG TAACTGGAGC AGATGGAGAC      2799
Ser Ser Gly Met Pro Leu Gly Ala Thr Gly Leu
        880                 885

CCCTTGGGGA GCAGGCTCGG GCTCCCCAGC CCCATCCCAA ACCCTTCAGT GCCAAAAACA      2859

ACAACAAAAT AGAAAGCGCA ACCACCACCA ACCACTGCGA CCACAAGAAG GATGATTCAA      2919

CAGGTTTTCC TGAAGAATTG AAAAACCATT TTGCTGTCCC TTTTCCTTTT TTGATGTTCT      2979

TTCACCCTTT TCTGTTTGCT AAGTGAGGAT GAAAAAATAA CACTGTACTG CAATAAGGGG      3039

AGAGTAACCC TGTCTAATGA AACCTGTGTC TCTGAGAGTA GAGTCACTGG AACACTAATG      3099

AGGAAACTGC ACTGTTTTAT TTTAATTCAG TTGTTAGTGT GTCTTAGTGT GTGCAATTTT      3159

TTTTCTTACT AATATCCATG GTTTGCAGGT TCTGTTAGGC CCTTTCCTTC TCCTGGAATT      3219

C                                                                      3220
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(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 906 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

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Met Gln His Ile Phe Ala Phe Phe Cys Thr Gly Phe Leu Gly Ala Val
-18         -15                 -10                  -5

Val Gly Ala Asn Phe Pro Asn Asn Ile Gln Ile Gly Gly Leu Phe Pro
        1                   5                  10

Asn Gln Gln Ser Gln Glu His Ala Ala Phe Arg Phe Ala Leu Ser Gln
 15                  20                  25                  30

Leu Thr Glu Pro Pro Lys L u Leu Pro Gln Ile Asp Ile Val Asn Ile
                35                  40                  45
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Ser Asp Thr Phe Glu Met Thr Tyr Arg Phe Cys Ser Gln Phe Ser Lys
            50                  55                  60

Gly Val Tyr Ala Ile Phe Gly Phe Tyr Glu Arg Arg Thr Val Asn Met
        65                  70                  75

Leu Thr Ser Phe Cys Gly Ala Leu His Val Cys Phe Ile Thr Pro Ser
    80                  85                  90

Phe Pro Val Asp Thr Ser Asn Gln Phe Val Leu Gln Leu Arg Pro Glu
 95                 100                 105                 110

Leu Gln Asp Ala Leu Ile Ser Ile Ile Asp His Tyr Lys Trp Gln Lys
                115                 120                 125

Phe Val Tyr Ile Tyr Asp Ala Asp Arg Gly Leu Ser Val Leu Gln Lys
            130                 135                 140

Val Leu Asp Thr Ala Ala Glu Lys Asn Trp Gln Val Thr Ala Val Asn
        145                 150                 155

Ile Leu Thr Thr Thr Glu Glu Gly Tyr Arg Met Leu Phe Gln Asp Leu
    160                 165                 170

Glu Lys Lys Lys Glu Arg Leu Val Val Val Asp Cys Glu Ser Glu Arg
175                 180                 185                 190

Leu Asn Ala Ile Leu Gly Gln Ile Ile Lys Leu Glu Lys Asn Gly Ile
                195                 200                 205

Gly Tyr His Tyr Ile Leu Ala Asn Leu Gly Phe Met Asp Ile Asp Leu
            210                 215                 220

Asn Lys Phe Lys Glu Ser Gly Ala Asn Val Thr Gly Phe Gln Leu Val
        225                 230                 235

Asn Tyr Thr Asp Thr Ile Pro Ala Lys Ile Met Gln Gln Trp Lys Asn
    240                 245                 250

Ser Asp Ala Arg Asp His Thr Arg Val Asp Trp Lys Arg Pro Lys Tyr
255                 260                 265                 270

Thr Ser Ala Leu Thr Tyr Asp Gly Val Lys Val Met Ala Glu Ala Phe
                275                 280                 285

Gln Ser Leu Arg Arg Gln Arg Ile Asp Ile Ser Arg Arg Gly Asn Ala
            290                 295                 300

Gly Asp Cys Leu Ala Asn Pro Ala Val Pro Trp Gly Gln Gly Ile Asp
        305                 310                 315

Ile Gln Arg Ala Leu Gln Gln Val Arg Phe Glu Gly Leu Thr Gly Asn
    320                 325                 330

Val Gln Phe Asn Glu Lys Gly Arg Arg Thr Asn Tyr Thr Leu His Val
335                 340                 345                 350

Ile Glu Met Lys His Asp Gly Ile Arg Lys Ile Gly Tyr Trp Asn Glu
                355                 360                 365

Asp Asp Lys Phe Val Pro Ala Ala Thr Asp Ala Gln Ala Gly Gly Asp
            370                 375                 380

Asn Ser Ser Val Gln Asn Arg Thr Tyr Ile Val Thr Thr Ile Leu Glu
        385                 390                 395
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Asp Pro Tyr Val Met Leu Lys Lys Asn Ala Asn Gln Phe Glu Gly Asn
    400                 405                 410

Asp Arg Tyr Glu Gly Tyr Cys Val Glu Leu Ala Ala Glu Ile Ala Lys
415                 420                 425                 430

His Val Gly Tyr Ser Tyr Arg Leu Glu Ile Val Ser Asp Gly Lys Tyr
                435                 440                 445

Gly Ala Arg Asp Pro Asp Thr Lys Ala Trp Asn Gly Met Val Gly Glu
            450                 455                 460

Leu Val Tyr Gly Arg Ala Asp Val Ala Val Ala Pro Leu Thr Ile Thr
        465                 470                 475

Leu Val Arg Glu Glu Val Ile Asp Phe Ser Lys Pro Phe Met Ser Leu
    480                 485                 490

Gly Ile Ser Ile Met Ile Lys Lys Pro Gln Lys Ser Lys Pro Gly Val
495                 500                 505                 510

Phe Ser Phe Leu Asp Pro Leu Ala Tyr Glu Ile Trp Met Cys Ile Val
                515                 520                 525

Phe Ala Tyr Ile Gly Val Ser Val Val Leu Phe Leu Val Ser Arg Phe
            530                 535                 540

Ser Pro Tyr Glu Trp His Ser Glu Glu Phe Glu Glu Gly Arg Asp Gln
        545                 550                 555

Thr Thr Ser Asp Gln Ser Asn Glu Phe Gly Ile Phe Asn Ser Leu Trp
    560                 565                 570

Phe Ser Leu Gly Ala Phe Met Gln Gln Gly Cys Asp Ile Ser Pro Arg
575                 580                 585                 590

Ser Leu Ser Gly Arg Ile Val Gly Gly Val Trp Trp Phe Phe Thr Leu
                595                 600                 605

Ile Ile Ile Ser Ser Tyr Thr Ala Asn Leu Ala Ala Phe Leu Thr Val
            610                 615                 620

Glu Arg Met Val Ser Pro Ile Glu Ser Ala Glu Asp Leu Ala Asn Glu
        625                 630                 635

Thr Glu Ile Ala Tyr Gly Thr Leu Glu Ala Gly Ser Thr Lys Glu Phe
    640                 645                 650

Phe Arg Arg Ser Lys Ile Ala Val Phe Glu Lys Met Trp Thr Tyr Met
655                 660                 665                 670

Lys Ser Ala Glu Pro Ser Val Phe Val Arg Thr Thr Glu Glu Gly Met
                675                 680                 685

Ile Arg Val Arg Lys Ser Lys Gly Lys Tyr Ala Tyr Leu Leu Glu Ser
            690                 695                 700

Thr Met Asn Glu Tyr Ile Glu Gln Arg Lys Pro Cys Asp Thr Met Lys
        705                 710                 715

Val Gly Gly Asn Leu Asp Ser Lys Gly Tyr Gly Ile Ala Thr Pro Lys
    720                 725                 730

Gly Ser Ala Leu Arg Gly Pro Val Asn Leu Ala Val Leu Lys L u Ser
735                 740                 745                 750
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Glu Gln Gly Val Leu Asp Lys Leu Lys Ser Lys Trp Trp Tyr Asp Lys
                755                 760                 765

Gly Glu Cys Gly Ser Lys Asp Ser Gly Ser Lys Asp Lys Thr Ser Ala
            770                 775                 780

Leu Ser Leu Ser Asn Val Ala Gly Val Phe Tyr Ile Leu Ile Gly Gly
        785                 790                 795

Leu Gly Leu Ala Met Leu Val Ala Leu Ile Glu Phe Cys Tyr Lys Ser
    800                 805                 810

Arg Ser Glu Ser Lys Arg Met Lys Gly Phe Cys Leu Ile Pro Gln Gln
815                 820                 825                 830

Ser Ile Asn Glu Ala Ile Arg Thr Ser Thr Leu Pro Arg Asn Ser Gly
                835                 840                 845

Ala Gly Ala Ser Ser Gly Gly Ser Gly Glu Asn Gly Arg Val Val Ser
            850                 855                 860

His Asp Phe Pro Lys Ser Met Gln Ser Ile Pro Cys Met Ser His Ser
        865                 870                 875

Ser Gly Met Pro Leu Gly Ala Thr Gly Leu
    880                 885
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(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 3407 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 315..2966

(ix) FEATURE:
(A) NAME/KEY: sig_peptide
(B) LOCATION: 315..374

(ix) FEATURE:
(A) NAME/KEY: mat_peptide
(B) LOCATION: 375..2966

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

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GAATTCCGTG AGTGCATGGG AGGGTGCTGA ATATTCCGAG ACACTGGGAC CACAGCGGCA      60

GCTCCGCTGA AAACTGCATT CAGCCAGTCC TCCGGACTTC TGGAGCGGGG ACAGGGCGCA     120

GGGCATCAGC AGCCACCAGC AGGACCTGGG AAATAGGGAT TCTTCTGCCT CCACTTCAGG     180

TTTTAGCAGC TTGGTGCTAA ATTGCTGTCT CAAAATGCAG AGGATCTAAT TTGCAGAGGA     240

AAACAGCCAA AGAAGGAAGA GGAGGAAAAG GAAAAAAAAA GGGGTATATT GTGGATGCTC     300

TACTTTTCTT GGAA ATG CAA AAG ATT ATG CAT ATT TCT GTC CTC CTT TCT       350
                Met Gln Lys Ile Met His Il  Ser Val Leu Leu Ser
                -20                 -15                 -10
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CCT GTT TTA TGG GGA CTG ATT TTT GGT GTC TCT TCT AAC AGC ATA CAG        398
Pro Val Leu Trp Gly Leu Ile Phe Gly Val Ser Ser Asn Ser Ile Gln
             -5                  1               5

ATA GGG GGG CTA TTT CCT AGG GGC GCC GAT CAA GAA TAC AGT GCA TTT        446
Ile Gly Gly Leu Phe Pro Arg Gly Ala Asp Gln Glu Tyr Ser Ala Phe
     10                  15                  20

CGA GTA GGG ATG GTT CAG TTT TCC ACT TCG GAG TTC AGA CTG ACA CCC        494
Arg Val Gly Met Val Gln Phe Ser Thr Ser Glu Phe Arg Leu Thr Pro
 25                  30                  35                   40

CAC ATC GAC AAT TTG GAG GTG GCA AAC AGC TTC GCA GTC ACT AAT GCT        542
His Ile Asp Asn Leu Glu Val Ala Asn Ser Phe Ala Val Thr Asn Ala
                 45                  50                  55

TTC TGC TCC CAG TTT TCG AGA GGA GTC TAT GCT ATT TTT GGA TTT TAT        590
Phe Cys Ser Gln Phe Ser Arg Gly Val Tyr Ala Ile Phe Gly Phe Tyr
             60                  65                  70

GAC AAG AAG TCT GTA AAT ACC ATC ACA TCA TTT TGC GGA ACA CTC CAC        638
Asp Lys Lys Ser Val Asn Thr Ile Thr Ser Phe Cys Gly Thr Leu His
         75                  80                  85

GTC TCC TTC ATC ACT CCC AGC TTC CCA ACA GAT GGC ACA CAT CCA TTT        686
Val Ser Phe Ile Thr Pro Ser Phe Pro Thr Asp Gly Thr His Pro Phe
     90                  95                 100

GTC ATT CAG ATG AGA CCC GAC CTC AAA GGA GCT CTC CTT AGC TTG ATT        734
Val Ile Gln Met Arg Pro Asp Leu Lys Gly Ala Leu Leu Ser Leu Ile
105                 110                 115                 120

GAA TAC TAT CAA TGG GAC AAG TTT GCA TAC CTC TAT GAC AGT GAC AGA        782
Glu Tyr Tyr Gln Trp Asp Lys Phe Ala Tyr Leu Tyr Asp Ser Asp Arg
                125                 130                 135

GGC TTA TCA ACA CTG CAA GCT GTG CTG GAT TCT GCT GCT GAA AAG AAA        830
Gly Leu Ser Thr Leu Gln Ala Val Leu Asp Ser Ala Ala Glu Lys Lys
            140                 145                 150

TGG CAA GTG ACT GCT ATC AAT GTG GGA AAC ATT AAC AAT GAC AAG AAA        878
Trp Gln Val Thr Ala Ile Asn Val Gly Asn Ile Asn Asn Asp Lys Lys
        155                 160                 165

GAT GAG ATG TAC CGA TCA CTT TTT CAA GAT CTG GAG TTA AAA AAG GAA        926
Asp Glu Met Tyr Arg Ser Leu Phe Gln Asp Leu Glu Leu Lys Lys Glu
    170                 175                 180

CGG CGT GTA ATT CTG GAC TGT GAA AGG GAT AAA GTA AAC GAC ATT GTA        974
Arg Arg Val Ile Leu Asp Cys Glu Arg Asp Lys Val Asn Asp Ile Val
185                 190                 195                 200

GAC CAG GTT ATT ACC ATT GGA AAA CAC GTT AAA GGG TAC CAC TAC ATC       1022
Asp Gln Val Ile Thr Ile Gly Lys His Val Lys Gly Tyr His Tyr Ile
                205                 210                 215

ATT GCA AAT CTG GGA TTT ACT GAT GGA GAC CTA TTA AAA ATC CAG TTT       1070
Ile Ala Asn Leu Gly Phe Thr Asp Gly Asp Leu Leu Lys Ile Gln Phe
            220                 225                 230

GGA GGT GCA AAT GTC TCT GGA TTT CAG ATA GTG GAC TAT GAT GAT TCG       1118
Gly Gly Ala Asn Val Ser Gly Phe Gln Ile Val Asp Tyr Asp Asp Ser
        235                 240                 245

TTG GTA TCT AAA TTT ATA GAA AGA TGG TCA ACA CTG GAA GAA AAA GAA       1166
Leu Val Ser Lys Phe Ile Glu Arg Trp Ser Thr Leu Glu Glu Lys Glu
    250                 255                 260
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TAC CCT GGA GCT CAC ACA ACA ACA ATT AAG TAT ACT TCT GCT CTG ACC     1214
Tyr Pro Gly Ala His Thr Thr Thr Ile Lys Tyr Thr Ser Ala Leu Thr
265                 270                 275                 280

TAT GAT GCC GTT CAA GTG ATG ACT GAA GCC TTC CGC AAC CTA AGG AAG     1262
Tyr Asp Ala Val Gln Val Met Thr Glu Ala Phe Arg Asn Leu Arg Lys
                285                 290                 295

CAA AGA ATT GAA ATC TCC CGA AGG GGG AAT GCA GGA GAC TGT CTG GCA     1310
Gln Arg Ile Glu Ile Ser Arg Arg Gly Asn Ala Gly Asp Cys Leu Ala
            300                 305                 310

AAC CCA GCA GTG CCC TGG GGA CAA GGT GTA GAA ATA GAA AGG GCC CTC     1358
Asn Pro Ala Val Pro Trp Gly Gln Gly Val Glu Ile Glu Arg Ala Leu
        315                 320                 325

AAA CAG GTT CAG GTT GAA GGT CTC TCA GGA AAT ATA AAG TTT GAC CAG     1406
Lys Gln Val Gln Val Glu Gly Leu Ser Gly Asn Ile Lys Phe Asp Gln
    330                 335                 340

AAT GGA AAA AGA ATA AAC TAT ACA ATT AAC ATC ATG GAG CTC AAA ACT     1454
Asn Gly Lys Arg Ile Asn Tyr Thr Ile Asn Ile Met Glu Leu Lys Thr
345                 350                 355                 360

AAT GGG CCC CGG AAG ATT GGC TAC TGG AGT GAA GTG GAC AAA ATG GTT     1502
Asn Gly Pro Arg Lys Ile Gly Tyr Trp Ser Glu Val Asp Lys Met Val
                365                 370                 375

GTT ACC CTT ACT GAG CTC CCT TCT GGA AAT GAC ACC TCT GGG CTT GAG     1550
Val Thr Leu Thr Glu Leu Pro Ser Gly Asn Asp Thr Ser Gly Leu Glu
            380                 385                 390

AAT AAG ACT GTT GTT GTC ACC ACA ATT TTG GAA TCT CCG TAT GTT ATG     1598
Asn Lys Thr Val Val Val Thr Thr Ile Leu Glu Ser Pro Tyr Val Met
        395                 400                 405

ATG AAG AAA AAT CAT GAA ATG CTT GAA GGC AAT GAG CGC TAT GAG GGC     1646
Met Lys Lys Asn His Glu Met Leu Glu Gly Asn Glu Arg Tyr Glu Gly
    410                 415                 420

TAC TGT GTT GAC CTG GCT GCA GAA ATC GCC AAA CAT TGT GGG TTC AAG     1694
Tyr Cys Val Asp Leu Ala Ala Glu Ile Ala Lys His Cys Gly Phe Lys
425                 430                 435                 440

TAC AAG TTG ACA ATT GTT GGT GAT GGC AAG TAT GGG GCC AGG GAT GCA     1742
Tyr Lys Leu Thr Ile Val Gly Asp Gly Lys Tyr Gly Ala Arg Asp Ala
                445                 450                 455

GAC ACG AAA ATT TGG AAT GGG ATG GTT GGA GAA CTT GTA TAT GGG AAA     1790
Asp Thr Lys Ile Trp Asn Gly Met Val Gly Glu Leu Val Tyr Gly Lys
            460                 465                 470

GCT GAT ATT GCA ATT GCT CCA TTA ACT ATT ACC CTT GTG AGA GAA GAG     1838
Ala Asp Ile Ala Ile Ala Pro Leu Thr Ile Thr Leu Val Arg Glu Glu
        475                 480                 485

GTG ATT GAC TTC TCA AAG CCC TTC ATG AGC CTC GGG ATA TCT ATC ATG     1886
Val Ile Asp Phe Ser Lys Pro Phe Met Ser Leu Gly Ile Ser Ile Met
    490                 495                 500

ATC AAG AAG CCT CAG AAG TCC AAA CCA GGA GTG TTT TCC TTT CTT GAT     1934
Ile Lys Lys Pro Gln Lys Ser Lys Pro Gly Val Phe Ser Phe Leu Asp
505                 510                 515                 520

CCT TTA GCC TAT GAG ATC TGG ATG TGC ATT GTT TTT GCC TAC ATT GGG     1982
Pro Leu Ala Tyr Glu Ile Trp Met Cys Ile Val Phe Ala Tyr Ile Gly
                525                 530                 535
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GTC AGT GTA GTT TTA TTC CTG GTC AGC AGA TTT AGC CCC TAC GAG TGG      2030
Val Ser Val Val L u Phe Leu Val Ser Arg Phe Ser Pro Tyr Glu Trp
            540                 545                 550

CAC ACT GAG GAG TTT GAA GAT GGA AGA GAA ACA CAA AGT AGT GAA TCA      2078
His Thr Glu Glu Phe Glu Asp Gly Arg Glu Thr Gln Ser Ser Glu Ser
        555                 560                 565

ACT AAT GAA TTT GGG ATT TTT AAT AGT CTC TGG TTT TCC TTG GGT GCC      2126
Thr Asn Glu Phe Gly Ile Phe Asn Ser Leu Trp Phe Ser Leu Gly Ala
    570                 575                 580

TTT ATG CGG CAA GGA TGC GAT ATT TCG CCA AGA TCC CTC TCT GGG CGC      2174
Phe Met Arg Gln Gly Cys Asp Ile Ser Pro Arg Ser Leu Ser Gly Arg
585                 590                 595                 600

ATT GTT GGA GGT GTG TGG TGG TTC TTT ACC CTG ATC ATA ATC TCC TCC      2222
Ile Val Gly Gly Val Trp Trp Phe Phe Thr Leu Ile Ile Ile Ser Ser
                605                 610                 615

TAC ACG GCT AAC TTA GCT GCC TTC CTG ACT GTA GAG AGG ATG GTG TCT      2270
Tyr Thr Ala Asn Leu Ala Ala Phe Leu Thr Val Glu Arg Met Val Ser
            620                 625                 630

CCC ATC GAA AGT GCT GAG GAT CTT TCT AAG CAA ACA GAA ATT GCT TAT      2318
Pro Ile Glu Ser Ala Glu Asp Leu Ser Lys Gln Thr Glu Ile Ala Tyr
        635                 640                 645

GGA ACA TTA GAC TCT GGC TCC ACT AAA GAG TTT TTC AGG AGA TCT AAA      2366
Gly Thr Leu Asp Ser Gly Ser Thr Lys Glu Phe Phe Arg Arg Ser Lys
    650                 655                 660

ATT GCA GTG TTT GAT AAA ATG TGG ACC TAC ATG CGG AGT GCG GAG CCC      2414
Ile Ala Val Phe Asp Lys Met Trp Thr Tyr Met Arg Ser Ala Glu Pro
665                 670                 675                 680

TCT GTG TTT GTG AGG ACT ACG GCC GAA GGG GTG GCT AGA GTG CGG AAG      2462
Ser Val Phe Val Arg Thr Thr Ala Glu Gly Val Ala Arg Val Arg Lys
                685                 690                 695

TCC AAA GGG AAA TAT GCC TAC TTG TTG GAG TCC ACG ATG AAC GAG TAC      2510
Ser Lys Gly Lys Tyr Ala Tyr Leu Leu Glu Ser Thr Met Asn Glu Tyr
            700                 705                 710

ATT GAG CAA AGG AAG CCT TGC GAC ACC ATG AAA GTT GGT GGA AAC CTG      2558
Ile Glu Gln Arg Lys Pro Cys Asp Thr Met Lys Val Gly Gly Asn Leu
        715                 720                 725

GAT TCC AAA GGC TAT GGC ATC GCA ACA CCT AAA GGA TCC TCA TTA GGA      2606
Asp Ser Lys Gly Tyr Gly Ile Ala Thr Pro Lys Gly Ser Ser Leu Gly
    730                 735                 740

ACC CCA GTA AAT CTT GCA GTA TTG AAA CTC AGT GAG CAA GGC GTC TTA      2654
Thr Pro Val Asn Leu Ala Val Leu Lys Leu Ser Glu Gln Gly Val Leu
745                 750                 755                 760

GAC AAG CTG AAA AAC AAA TGG TGG TAC GAT AAA GGT GAA TGT GGA GCC      2702
Asp Lys Leu Lys Asn Lys Trp Trp Tyr Asp Lys Gly Glu Cys Gly Ala
                765                 770                 775

AAG GAC TCT GGA AGT AAG GAA AAG ACC AGT GCC CTC AGT CTG AGC AAC      2750
Lys Asp Ser Gly Ser Lys Glu Lys Thr Ser Ala Leu Ser Leu Ser Asn
            780                 785                 790

GTT GCT GGA GTA TTC TAC ATC CTT GTC GGG GGC CTT GGT TTG GCA ATG      2798
Val Ala Gly Val Phe Tyr Ile Leu Val Gly Gly Leu Gly Leu Ala Met
        795                 800                 805
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CTG GTG GCT TTG ATT GAG TTC TGT TAC AAG TCA AGG GCC GAG GCG AAA      2846
Leu Val Ala Leu Ile Glu Phe Cys Tyr Lys Ser Arg Ala Glu Ala Lys
    810                 815                 820

CGA ATG AAG GTG GCA AAG AAT GCA CAG AAT ATT AAC CCA TCT TCC TCG      2894
Arg Met Lys Val Ala Lys Asn Ala Gln Asn Ile Asn Pro Ser Ser Ser
825                 830                 835                 840

CAG AAT TCA CAG AAT TTT GCA ACT TAT AAG GAA GGT TAC AAC GTA TAT      2942
Gln Asn Ser Gln Asn Phe Ala Thr Tyr Lys Glu Gly Tyr Asn Val Tyr
                845                 850                 855

GGC ATC GAA AGT GTT AAA ATT TAGGGGATGA CCTTGAATGA TGCCATGAGG         2993
Gly Ile Glu Ser Val Lys Ile
            860

AACAAGGCAA GGCTGTCAAT TACAGGAAGT ACTGGAGAAA ATGGACGTGT TATGACTCCA    3053

GAATTTCCCA AAGCNGTGCA TGCTGTCCCT TACGTGAGTC CTGGCATGGG AATGAATGTC    3113

AGTGTGACTG ATCTCTCGTG ATTGATAAGA ACCTTTTGAG TGCCTTACAC AATGGTTTTC    3173

TTGTGTGTTT ATTGTCAAAG TGGTGAGAGG CATCCAGTAT CTTGAAGACT TTTCTTTCAG    3233

CCAAGAATTC TTAAATATGT GGAGTTCATC TTGAATTGTA AGGAATGATT AATTAAAACA    3293

CAACATCTTT TTCTACTCGA GTTACAGACA AAGCGTGGTG GACATGCACA GCTAACATGG    3353

AAGTACTATA ATTTACCTGA AGTCTTTGTA CAGACAACAA ACCTGTTTCT GCAG          3407
```

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 883 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

```
Met Gln Lys Ile Met His Ile Ser Val Leu Leu Ser Pro Val Leu Trp
-20                 -15                 -10                  -5

Gly Leu Ile Phe Gly Val Ser Ser Asn Ser Ile Gln Ile Gly Gly Leu
                  1               5                  10

Phe Pro Arg Gly Ala Asp Gln Glu Tyr Ser Ala Phe Arg Val Gly Met
         15                  20                  25

Val Gln Phe Ser Thr Ser Glu Phe Arg Leu Thr Pro His Ile Asp Asn
    30                  35                  40

Leu Glu Val Ala Asn Ser Phe Ala Val Thr Asn Ala Phe Cys Ser Gln
 45                  50                  55                  60

Phe Ser Arg Gly Val Tyr Ala Ile Phe Gly Phe Tyr Asp Lys Lys Ser
                65                  70                  75

Val Asn Thr Ile Thr Ser Phe Cys Gly Thr Leu His Val Ser Phe Ile
            80                  85                  90

Thr Pro S r Phe Pro Thr Asp Gly Thr His Pro Phe Val Ile Gln Met
         95                 100                 105
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Arg Pro Asp Leu Lys Gly Ala Leu Leu Ser Leu Ile Glu Tyr Tyr Gln
    110                 115                 120

Trp Asp Lys Phe Ala Tyr Leu Tyr Asp Ser Asp Arg Gly Leu Ser Thr
125                 130                 135                 140

Leu Gln Ala Val Leu Asp Ser Ala Ala Glu Lys Lys Trp Gln Val Thr
                145                 150                 155

Ala Ile Asn Val Gly Asn Ile Asn Asn Asp Lys Lys Asp Glu Met Tyr
            160                 165                 170

Arg Ser Leu Phe Gln Asp Leu Glu Leu Lys Lys Glu Arg Arg Val Ile
        175                 180                 185

Leu Asp Cys Glu Arg Asp Lys Val Asn Asp Ile Val Asp Gln Val Ile
    190                 195                 200

Thr Ile Gly Lys His Val Lys Gly Tyr His Tyr Ile Ile Ala Asn Leu
205                 210                 215                 220

Gly Phe Thr Asp Gly Asp Leu Leu Lys Ile Gln Phe Gly Gly Ala Asn
                225                 230                 235

Val Ser Gly Phe Gln Ile Val Asp Tyr Asp Asp Ser Leu Val Ser Lys
            240                 245                 250

Phe Ile Glu Arg Trp Ser Thr Leu Glu Glu Lys Glu Tyr Pro Gly Ala
        255                 260                 265

His Thr Thr Thr Ile Lys Tyr Thr Ser Ala Leu Thr Tyr Asp Ala Val
    270                 275                 280

Gln Val Met Thr Glu Ala Phe Arg Asn Leu Arg Lys Gln Arg Ile Glu
285                 290                 295                 300

Ile Ser Arg Arg Gly Asn Ala Gly Asp Cys Leu Ala Asn Pro Ala Val
                305                 310                 315

Pro Trp Gly Gln Gly Val Glu Ile Glu Arg Ala Leu Lys Gln Val Gln
            320                 325                 330

Val Glu Gly Leu Ser Gly Asn Ile Lys Phe Asp Gln Asn Gly Lys Arg
        335                 340                 345

Ile Asn Tyr Thr Ile Asn Ile Met Glu Leu Lys Thr Asn Gly Pro Arg
    350                 355                 360

Lys Ile Gly Tyr Trp Ser Glu Val Asp Lys Met Val Val Thr Leu Thr
365                 370                 375                 380

Glu Leu Pro Ser Gly Asn Asp Thr Ser Gly Leu Glu Asn Lys Thr Val
                385                 390                 395

Val Val Thr Thr Ile Leu Glu Ser Pro Tyr Val Met Met Lys Lys Asn
            400                 405                 410

His Glu Met Leu Glu Gly Asn Glu Arg Tyr Glu Gly Tyr Cys Val Asp
        415                 420                 425

Leu Ala Ala Glu Ile Ala Lys His Cys Gly Phe Lys Tyr Lys Leu Thr
    430                 435                 440

Ile Val Gly Asp Gly Lys Tyr Gly Ala Arg Asp Ala Asp Thr Lys Ile
445                 450                 455                 460
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Trp Asn Gly Met Val Gly Glu Leu Val Tyr Gly Lys Ala Asp Ile Ala
                465                 470                 475

Ile Ala Pro Leu Thr Ile Thr Leu Val Arg Glu Glu Val Ile Asp Phe
            480                 485                 490

Ser Lys Pro Phe Met Ser Leu Gly Ile Ser Ile Met Ile Lys Lys Pro
        495                 500                 505

Gln Lys Ser Lys Pro Gly Val Phe Ser Phe Leu Asp Pro Leu Ala Tyr
    510                 515                 520

Glu Ile Trp Met Cys Ile Val Phe Ala Tyr Ile Gly Val Ser Val Val
525                 530                 535                 540

Leu Phe Leu Val Ser Arg Phe Ser Pro Tyr Glu Trp His Thr Glu Glu
                545                 550                 555

Phe Glu Asp Gly Arg Glu Thr Gln Ser Ser Glu Ser Thr Asn Glu Phe
            560                 565                 570

Gly Ile Phe Asn Ser Leu Trp Phe Ser Leu Gly Ala Phe Met Arg Gln
        575                 580                 585

Gly Cys Asp Ile Ser Pro Arg Ser Leu Ser Gly Arg Ile Val Gly Gly
    590                 595                 600

Val Trp Trp Phe Phe Thr Leu Ile Ile Ile Ser Ser Tyr Thr Ala Asn
605                 610                 615                 620

Leu Ala Ala Phe Leu Thr Val Glu Arg Met Val Ser Pro Ile Glu Ser
                625                 630                 635

Ala Glu Asp Leu Ser Lys Gln Thr Glu Ile Ala Tyr Gly Thr Leu Asp
            640                 645                 650

Ser Gly Ser Thr Lys Glu Phe Phe Arg Arg Ser Lys Ile Ala Val Phe
        655                 660                 665

Asp Lys Met Trp Thr Tyr Met Arg Ser Ala Glu Pro Ser Val Phe Val
    670                 675                 680

Arg Thr Thr Ala Glu Gly Val Ala Arg Val Arg Lys Ser Lys Gly Lys
685                 690                 695                 700

Tyr Ala Tyr Leu Leu Glu Ser Thr Met Asn Glu Tyr Ile Glu Gln Arg
                705                 710                 715

Lys Pro Cys Asp Thr Met Lys Val Gly Gly Asn Leu Asp Ser Lys Gly
            720                 725                 730

Tyr Gly Ile Ala Thr Pro Lys Gly Ser Ser Leu Gly Thr Pro Val Asn
        735                 740                 745

Leu Ala Val Leu Lys Leu Ser Glu Gln Gly Val Leu Asp Lys Leu Lys
    750                 755                 760

Asn Lys Trp Trp Tyr Asp Lys Gly Glu Cys Gly Ala Lys Asp Ser Gly
765                 770                 775                 780

Ser Lys Glu Lys Thr Ser Ala Leu Ser Leu Ser Asn Val Ala Gly Val
                785                 790                 795

Ph  Tyr Ile L u Val Gly Gly Leu Gly Leu Ala Met Leu Val Ala Leu
            800                 805                 810
```

```
Ile Glu Phe Cys Tyr Lys Ser Arg Ala Glu Ala Lys Arg Met Lys Val
        815                 820                 825

Ala Lys Asn Ala Gln Asn Ile Asn Pro Ser Ser Ser Gln Asn Ser Gln
    830                 835                 840

Asn Phe Ala Thr Tyr Lys Glu Gly Tyr Asn Val Tyr Gly Ile Glu Ser
845                 850                 855                 860

Val Lys Ile
```

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2761 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: sig_peptide
(B) LOCATION: 79..144

(ix) FEATURE:
(A) NAME/KEY: mat_peptide
(B) LOCATION: 145..2745

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 79..2745

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

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GAATTCCTGA CGACTCCTGA GTTGCGCCCA TGCTCTTGTC AGCTTCGTTT TAGGCGTAGC      60

ATGGCCAGGC AGAAGAAA ATG GGG CAA AGC GTG CTC CGG GCG GTC TTC TTT       111
                    Met Gly Gln Ser Val Leu Arg Ala Val Phe Phe
                    -22     -20                 -15

TTA GTC CTG GGG CTT TTG GGT CAT TCT CAC GGA GGA TTC CCC AAC ACC       159
Leu Val Leu Gly Leu Leu Gly His Ser His Gly Gly Phe Pro Asn Thr
    -10                 -5                   1                   5

ATC AGC ATA GGT GGA CTT TTC ATG AGA AAC ACA GTG CAG GAG CAC AGC       207
Ile Ser Ile Gly Gly Leu Phe Met Arg Asn Thr Val Gln Glu His Ser
                10                  15                  20

GCT TTC CGC TTT GCC GTG CAG TTA TAC AAC ACC AAC CAG AAC ACC ACC       255
Ala Phe Arg Phe Ala Val Gln Leu Tyr Asn Thr Asn Gln Asn Thr Thr
            25                  30                  35

GAG AAG CCC TTC CAT TTG AAT TAC CAC GTA GAT CAC TTG GAT TCC TCC       303
Glu Lys Pro Phe His Leu Asn Tyr His Val Asp His Leu Asp Ser Ser
        40                  45                  50

AAT AGT TTT TCC GTG ACA AAT GCT TTC TGC TCC CAG TTC TCG AGA GGG       351
Asn Ser Phe Ser Val Thr Asn Ala Phe Cys Ser Gln Phe Ser Arg Gly
    55                  60                  65

GTG TAT GCC ATC TTT GGA TTC TAT GAC CAG ATG TCA ATG AAC ACC CTG       399
Val Tyr Ala Ile Phe Gly Phe Tyr Asp Gln Met Ser Met Asn Thr Leu
70                  75                  80                  85
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ACC TCC TTC TGT GGG GCC CTG CAC ACA TCC TTT GTT ACG CCT AGC TTC 447
Thr Ser Phe Cys Gly Ala Leu His Thr Ser Phe Val Thr Pro Ser Phe
90 95 100

CCC ACT GAC GCA GAT GTG CAG TTT GTC ATC CAG ATG CGC CCA GCC TTG 495
Pro Thr Asp Ala Asp Val Gln Phe Val Ile Gln Met Arg Pro Ala Leu
105 110 115

AAG GGC GCT ATT CTG AGT CTT CTG GGT CAT TAC AAG TGG GAG AAG TTT 543
Lys Gly Ala Ile Leu Ser Leu Leu Gly His Tyr Lys Trp Glu Lys Phe
120 125 130

GTG TAC CTC TAT GAC ACA GAA CGA GGA TTT TCC ATC CTC CAA GCG ATT 591
Val Tyr Leu Tyr Asp Thr Glu Arg Gly Phe Ser Ile Leu Gln Ala Ile
135 140 145

ATG GAA GCA GCA GTG CAA AAC AAC TGG CAA GTA ACA GCA AGG TCT GTG 639
Met Glu Ala Ala Val Gln Asn Asn Trp Gln Val Thr Ala Arg Ser Val
150 155 160 165

GGA AAC ATA AAG GAC GTC CAA GAA TTC AGG CGC ATC ATT GAA GAA ATG 687
Gly Asn Ile Lys Asp Val Gln Glu Phe Arg Arg Ile Ile Glu Glu Met
170 175 180

GAC AGG AGG CAG GAA AAG CGA TAC TTG ATT GAC TGC GAA GTC GAA AGG 735
Asp Arg Arg Gln Glu Lys Arg Tyr Leu Ile Asp Cys Glu Val Glu Arg
185 190 195

ATT AAC ACA ATT TTG GAA CAG GTT GTG ATC CTA GGG AAA CAC TCA AGA 783
Ile Asn Thr Ile Leu Glu Gln Val Val Ile Leu Gly Lys His Ser Arg
200 205 210

GGT TAT CAC TAC ATG CTC GCT AAC CTG GGT TTT ACT GAT ATT TTA CTG 831
Gly Tyr His Tyr Met Leu Ala Asn Leu Gly Phe Thr Asp Ile Leu Leu
215 220 225

GAA AGA GTC ATG CAT GGG GGA GCC AAC ATT ACA GGT TTC CAG ATT GTC 879
Glu Arg Val Met His Gly Gly Ala Asn Ile Thr Gly Phe Gln Ile Val
230 235 240 245

AAC AAT GAA AAC CCT ATG GTT CAG CAG TTC ATA CAG CGC TGG GTG AGG 927
Asn Asn Glu Asn Pro Met Val Gln Gln Phe Ile Gln Arg Trp Val Arg
250 255 260

CTG GAT GAA AGG GAA TTC CCT GAA GCC AAG AAT GCA CCA CTA AAG TAT 975
Leu Asp Glu Arg Glu Phe Pro Glu Ala Lys Asn Ala Pro Leu Lys Tyr
265 270 275

ACA TCT GCA TTG ACA CAC GAC GCA ATA CTG GTC ATA GCA GAA GCT TTC 1023
Thr Ser Ala Leu Thr His Asp Ala Ile Leu Val Ile Ala Glu Ala Phe
280 285 290

CGC TAC CTG AGG AGG CAG CGA GTA GAT GTG TCC CGG AGA GGA AGT GCT 1071
Arg Tyr Leu Arg Arg Gln Arg Val Asp Val Ser Arg Arg Gly Ser Ala
295 300 305

GGA GAC TGC TTA GCA AAT CCT GCT GTG CCC TGG AGT CAA GGA ATT GAT 1119
Gly Asp Cys Leu Ala Asn Pro Ala Val Pro Trp Ser Gln Gly Ile Asp
310 315 320 325

ATT GAG AGA GCT CTG AAA ATG GTG CAA GTA CAA GGA ATG ACT GGA AAT 1167
Ile Glu Arg Ala Leu Lys Met Val Gln Val Gln Gly Met Thr Gly Asn
330 335 340

ATT CAA TTT GAC ACT TAT GGA CGT AGG ACA AAT TAT ACC ATC GAT GTG 1215
Ile Gln Phe Asp Thr Tyr Gly Arg Arg Thr Asn Tyr Thr Ile Asp Val
345 350 355

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TAT GAA ATG AAA GTC AGT GGC TCT CGA AAA GCT GGC TAC TGG AAC GAG      1263
Tyr Glu Met Lys Val Ser Gly Ser Arg Lys Ala Gly Tyr Trp Asn Glu
        360                 365                 370

TAT GAA AGG TTT GTG CCT TTC TCA GAT CAG CAA ATC AGC AAT GAC AGT      1311
Tyr Glu Arg Phe Val Pro Phe Ser Asp Gln Gln Ile Ser Asn Asp Ser
    375                 380                 385

GCA TCC TCA GAG AAT CGG ACC ATA GTA GTG ACT ACC ATT CTG GAA TCA      1359
Ala Ser Ser Glu Asn Arg Thr Ile Val Val Thr Thr Ile Leu Glu Ser
390                 395                 400                 405

CCA TAT GTA ATG TAC AAG AAG AAC CAT GAG CAA CTG GAA GGA AAT GAA      1407
Pro Tyr Val Met Tyr Lys Lys Asn His Glu Gln Leu Glu Gly Asn Glu
                410                 415                 420

CGA TAT GAA GGC TAT TGT GTA GAC CTA GCC TAT GAA ATA GCC AAA CAT      1455
Arg Tyr Glu Gly Tyr Cys Val Asp Leu Ala Tyr Glu Ile Ala Lys His
            425                 430                 435

GTA AGG ATC AAA TAC AAA TTG TCC ATC GTT GGT GAC GGG AAA TAT GGT      1503
Val Arg Ile Lys Tyr Lys Leu Ser Ile Val Gly Asp Gly Lys Tyr Gly
        440                 445                 450

GCA AGG GAT CCA GAG ACT AAA ATA TGG AAC GGC ATG GTT GGG GAA CTT      1551
Ala Arg Asp Pro Glu Thr Lys Ile Trp Asn Gly Met Val Gly Glu Leu
    455                 460                 465

GTC TAT GGG AGA GCT GAT ATA GCT GTT GCT CCA CTC ACT ATA ACA TTG      1599
Val Tyr Gly Arg Ala Asp Ile Ala Val Ala Pro Leu Thr Ile Thr Leu
470                 475                 480                 485

GTC CGT GAA GAA GTC ATA GAT TTT TCA AAG CCA TTA ATG AGC CTG GGC      1647
Val Arg Glu Glu Val Ile Asp Phe Ser Lys Pro Leu Met Ser Leu Gly
                490                 495                 500

ATC TCC ATC ATG ATA AAG AAG CCT CAG AAA TCA AAA CCA GGC GTA TTC      1695
Ile Ser Ile Met Ile Lys Lys Pro Gln Lys Ser Lys Pro Gly Val Phe
            505                 510                 515

TCA TTT CTG GAT CCC CTG GCT TAT GAA ATC TGG ATG TGC ATT GTC TTT      1743
Ser Phe Leu Asp Pro Leu Ala Tyr Glu Ile Trp Met Cys Ile Val Phe
        520                 525                 530

GCT TAC ATT GGA GTC AGC GTA GTT CTT TTC CTA GTC AGC AGG TTC AGT      1791
Ala Tyr Ile Gly Val Ser Val Val Leu Phe Leu Val Ser Arg Phe Ser
    535                 540                 545

CCT TAT GAA TGG CAC TTG GAA GAC AAC AAT GAA GAA CCT CGT GAC CCA      1839
Pro Tyr Glu Trp His Leu Glu Asp Asn Asn Glu Glu Pro Arg Asp Pro
550                 555                 560                 565

CAA AGT CCT CCT GAT CCT CCA AAT GAA TTT GGA ATA TTT AAC AGT CTT      1887
Gln Ser Pro Pro Asp Pro Pro Asn Glu Phe Gly Ile Phe Asn Ser Leu
                570                 575                 580

TGG TTT TCC TTG GGT GCC TTT ATG CAG CAA GGA TGT GAT ATT TCT CCA      1935
Trp Phe Ser Leu Gly Ala Phe Met Gln Gln Gly Cys Asp Ile Ser Pro
            585                 590                 595

AGA TCA CTC TCC GGG CGC ATT GTT GGA GGG GTT TGG TGG TTC TTC ACC      1983
Arg Ser Leu Ser Gly Arg Ile Val Gly Gly Val Trp Trp Phe Phe Thr
        600                 605                 610

CTG ATC ATA ATT TCT TCC TAT ACT GCC AAT CTC GCT GCT TTC CTG ACT      2031
Leu Ile Ile Ile Ser Ser Tyr Thr Ala Asn Leu Ala Ala Phe Leu Thr
    615                 620                 625
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GTG GAG AGG ATG GTT TCT CCC ATA GAG AGT GCT GAA GAC TTA GCT AAA      2079
Val Glu Arg Met Val Ser Pro Ile Glu Ser Ala Glu Asp Leu Ala Lys
630                 635                      640                 645

CAG ACT GAA ATT GCA TAT GGG ACC CTG GAC TCC GGT TCA ACA AAA GAA      2127
Gln Thr Glu Ile Ala Tyr Gly Thr Leu Asp Ser Gly Ser Thr Lys Glu
                650                      655                 660

TTT TTC AGA AGA TCC AAA ATT GCT GTG TAC GAG AAA ATG TGG TCT TAC      2175
Phe Phe Arg Arg Ser Lys Ile Ala Val Tyr Glu Lys Met Trp Ser Tyr
            665                      670                 675

ATG AAA TCA GCG GAG CCA TCT GTG TTT ACC AAA ACA ACA GCA GAC GGA      2223
Met Lys Ser Ala Glu Pro Ser Val Phe Thr Lys Thr Thr Ala Asp Gly
        680                 685                      690

GTG GCC CGA GTG CGA AAG TCC AAG GGA AAG TTC GCC TTC CTG CTG GAG      2271
Val Ala Arg Val Arg Lys Ser Lys Gly Lys Phe Ala Phe Leu Leu Glu
    695                      700                 705

TCA ACC ATG AAT GAG TAC ATT GAG CAG AGA AAA CCA TGT GAT ACG ATG      2319
Ser Thr Met Asn Glu Tyr Ile Glu Gln Arg Lys Pro Cys Asp Thr Met
710                 715                      720                 725

AAA GTT GGT GGA AAT CTG GAT TCC AAA GGC TAT GGT GTG GCA ACC CCT      2367
Lys Val Gly Gly Asn Leu Asp Ser Lys Gly Tyr Gly Val Ala Thr Pro
                730                      735                 740

AAA GGC TCA GCA TTA GGA AAT GCT GTT AAC CTG GCA GTA TTA AAA CTG      2415
Lys Gly Ser Ala Leu Gly Asn Ala Val Asn Leu Ala Val Leu Lys Leu
            745                      750                 755

AAT GAG CAA GGC CTC TTG GAC AAA TTG AAA AAC AAA TGG TGG TAC GAC      2463
Asn Glu Gln Gly Leu Leu Asp Lys Leu Lys Asn Lys Trp Trp Tyr Asp
        760                 765                      770

AAA GGA GAG TGC GGC AGC GGG GGC GGT GAC TCC AAG GAC AAG ACC AGC      2511
Lys Gly Glu Cys Gly Ser Gly Gly Gly Asp Ser Lys Asp Lys Thr Ser
    775                      780                 785

GCT CTG AGC CTG AGC AAT GTG GCA GGC GTT TTC TAT ATA CTT GTC GGA      2559
Ala Leu Ser Leu Ser Asn Val Ala Gly Val Phe Tyr Ile Leu Val Gly
790                 795                      800                 805

GGT CTG GGG CTG GCC ATG ATG GTG GCT TTG ATA GAA TTC TGT TAC AAA      2607
Gly Leu Gly Leu Ala Met Met Val Ala Leu Ile Glu Phe Cys Tyr Lys
                810                      815                 820

TCA CGG GCA GAG TCC AAA CGC ATG AAA CTC ACA AAG AAC ACC CAA AAC      2655
Ser Arg Ala Glu Ser Lys Arg Met Lys Leu Thr Lys Asn Thr Gln Asn
            825                      830                 835

TTT AAG CCT GCT CCT GCC ACC AAC ACT CAG AAT TAT GCT ACA TAC AGA      2703
Phe Lys Pro Ala Pro Ala Thr Asn Thr Gln Asn Tyr Ala Thr Tyr Arg
        840                 845                      850

GAA GGC TAC AAC GTG TAT GGA ACA GAG AGT GTT AAG ATC TAGGGATCCC       2752
Glu Gly Tyr Asn Val Tyr Gly Thr Glu Ser Val Lys Ile
    855                      860                 865

TTGGAATTC                                                            2761
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(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 888 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

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Met Gly Gln Ser Val Leu Arg Ala Val Phe Phe Leu Val Leu Gly Leu
-22     -20                 -15                 -10

Leu Gly His Ser His Gly Gly Phe Pro Asn Thr Ile Ser Ile Gly Gly
     -5                   1                   5                  10

Leu Phe Met Arg Asn Thr Val Gln Glu His Ser Ala Phe Arg Phe Ala
                 15                  20                  25

Val Gln Leu Tyr Asn Thr Asn Gln Asn Thr Thr Glu Lys Pro Phe His
             30                  35                  40

Leu Asn Tyr His Val Asp His Leu Asp Ser Ser Asn Ser Phe Ser Val
         45                  50                  55

Thr Asn Ala Phe Cys Ser Gln Phe Ser Arg Gly Val Tyr Ala Ile Phe
     60                  65                  70

Gly Phe Tyr Asp Gln Met Ser Met Asn Thr Leu Thr Ser Phe Cys Gly
 75                  80                  85                  90

Ala Leu His Thr Ser Phe Val Thr Pro Ser Phe Pro Thr Asp Ala Asp
                 95                 100                 105

Val Gln Phe Val Ile Gln Met Arg Pro Ala Leu Lys Gly Ala Ile Leu
            110                 115                 120

Ser Leu Leu Gly His Tyr Lys Trp Glu Lys Phe Val Tyr Leu Tyr Asp
        125                 130                 135

Thr Glu Arg Gly Phe Ser Ile Leu Gln Ala Ile Met Glu Ala Ala Val
    140                 145                 150

Gln Asn Asn Trp Gln Val Thr Ala Arg Ser Val Gly Asn Ile Lys Asp
155                 160                 165                 170

Val Gln Glu Phe Arg Arg Ile Ile Glu Glu Met Asp Arg Arg Gln Glu
                175                 180                 185

Lys Arg Tyr Leu Ile Asp Cys Glu Val Glu Arg Ile Asn Thr Ile Leu
            190                 195                 200

Glu Gln Val Val Ile Leu Gly Lys His Ser Arg Gly Tyr His Tyr Met
        205                 210                 215

Leu Ala Asn Leu Gly Phe Thr Asp Ile Leu Leu Glu Arg Val Met His
    220                 225                 230

Gly Gly Ala Asn Ile Thr Gly Phe Gln Ile Val Asn Asn Glu Asn Pro
235                 240                 245                 250

Met Val Gln Gln Phe Ile Gln Arg Trp Val Arg Leu Asp Glu Arg Glu
                255                 260                 265

Phe Pro Glu Ala Lys Asn Ala Pro Leu Lys Tyr Thr Ser Ala Leu Thr
            270                 275                 280
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His Asp Ala Ile Leu Val Ile Ala Glu Ala Phe Arg Tyr Leu Arg Arg
        285                 290                 295

Gln Arg Val Asp Val Ser Arg Arg Gly Ser Ala Gly Asp Cys Leu Ala
    300                 305                 310

Asn Pro Ala Val Pro Trp Ser Gln Gly Ile Asp Ile Glu Arg Ala Leu
315                 320                 325                     330

Lys Met Val Gln Val Gln Gly Met Thr Gly Asn Ile Gln Phe Asp Thr
                335                 340                 345

Tyr Gly Arg Arg Thr Asn Tyr Thr Ile Asp Val Tyr Glu Met Lys Val
            350                 355                 360

Ser Gly Ser Arg Lys Ala Gly Tyr Trp Asn Glu Tyr Glu Arg Phe Val
        365                 370                 375

Pro Phe Ser Asp Gln Gln Ile Ser Asn Asp Ser Ala Ser Ser Glu Asn
    380                 385                 390

Arg Thr Ile Val Val Thr Thr Ile Leu Glu Ser Pro Tyr Val Met Tyr
395                 400                 405                     410

Lys Lys Asn His Glu Gln Leu Glu Gly Asn Glu Arg Tyr Glu Gly Tyr
                415                 420                 425

Cys Val Asp Leu Ala Tyr Glu Ile Ala Lys His Val Arg Ile Lys Tyr
            430                 435                 440

Lys Leu Ser Ile Val Gly Asp Gly Lys Tyr Gly Ala Arg Asp Pro Glu
        445                 450                 455

Thr Lys Ile Trp Asn Gly Met Val Gly Glu Leu Val Tyr Gly Arg Ala
    460                 465                 470

Asp Ile Ala Val Ala Pro Leu Thr Ile Thr Leu Val Arg Glu Glu Val
475                 480                 485                     490

Ile Asp Phe Ser Lys Pro Leu Met Ser Leu Gly Ile Ser Ile Met Ile
                495                 500                 505

Lys Lys Pro Gln Lys Ser Lys Pro Gly Val Phe Ser Phe Leu Asp Pro
            510                 515                 520

Leu Ala Tyr Glu Ile Trp Met Cys Ile Val Phe Ala Tyr Ile Gly Val
        525                 530                 535

Ser Val Val Leu Phe Leu Val Ser Arg Phe Ser Pro Tyr Glu Trp His
    540                 545                 550

Leu Glu Asp Asn Asn Glu Glu Pro Arg Asp Pro Gln Ser Pro Pro Asp
555                 560                 565                     570

Pro Pro Asn Glu Phe Gly Ile Phe Asn Ser Leu Trp Phe Ser Leu Gly
                575                 580                 585

Ala Phe Met Gln Gln Gly Cys Asp Ile Ser Pro Arg Ser Leu Ser Gly
            590                 595                 600

Arg Ile Val Gly Gly Val Trp Trp Phe Phe Thr Leu Ile Ile Ile Ser
        605                 610                 615

Ser Tyr Thr Ala Asn L u Ala Ala Phe Leu Thr Val Glu Arg Met Val
    620                 625                 630
```

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Ser Pro Ile Glu Ser Ala Glu Asp Leu Ala Lys Gln Thr Glu Ile Ala
635                 640                 645                 650

Tyr Gly Thr Leu Asp Ser Gly Ser Thr Lys Glu Phe Phe Arg Arg Ser
                655                 660                 665

Lys Ile Ala Val Tyr Glu Lys Met Trp Ser Tyr Met Lys Ser Ala Glu
            670                 675                 680

Pro Ser Val Phe Thr Lys Thr Thr Ala Asp Gly Val Ala Arg Val Arg
        685                 690                 695

Lys Ser Lys Gly Lys Phe Ala Phe Leu Leu Glu Ser Thr Met Asn Glu
    700                 705                 710

Tyr Ile Glu Gln Arg Lys Pro Cys Asp Thr Met Lys Val Gly Gly Asn
715                 720                 725                 730

Leu Asp Ser Lys Gly Tyr Gly Val Ala Thr Pro Lys Gly Ser Ala Leu
                735                 740                 745

Gly Asn Ala Val Asn Leu Ala Val Leu Lys Leu Asn Glu Gln Gly Leu
            750                 755                 760

Leu Asp Lys Leu Lys Asn Lys Trp Trp Tyr Asp Lys Gly Glu Cys Gly
        765                 770                 775

Ser Gly Gly Gly Asp Ser Lys Asp Lys Thr Ser Ala Leu Ser Leu Ser
    780                 785                 790

Asn Val Ala Gly Val Phe Tyr Ile Leu Val Gly Gly Leu Gly Leu Ala
795                 800                 805                 810

Met Met Val Ala Leu Ile Glu Phe Cys Tyr Lys Ser Arg Ala Glu Ser
                815                 820                 825

Lys Arg Met Lys Leu Thr Lys Asn Thr Gln Asn Phe Lys Pro Ala Pro
            830                 835                 840

Ala Thr Asn Thr Gln Asn Tyr Ala Thr Tyr Arg Glu Gly Tyr Asn Val
        845                 850                 855

Tyr Gly Thr Glu Ser Val Lys Ile
    860                 865
```

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 3070 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: sig_peptide
(B) LOCATION: 79..144

(ix) FEATURE:
(A) NAME/KEY: mat_peptide
(B) LOCATION: 145..2745

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 79..2745

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

```
GAATTCCTGA CGACTCCTGA GTTGCGCCCA TGCTCTTGTC AGCTTCGTTT TAGGCGTAGC      60

ATGGCCAGGC AGAAGAAA ATG GGG CAA AGC GTG CTC CGG GCG GTC TTC TTT       111
                    Met Gly Gln Ser Val Leu Arg Ala Val Phe Phe
                    -22     -20                 -15

TTA GTC CTG GGG CTT TTG GGT CAT TCT CAC GGA GGA TTC CCC AAC ACC       159
Leu Val Leu Gly Leu Leu Gly His Ser His Gly Gly Phe Pro Asn Thr
    -10                 -5                  1               5

ATC AGC ATA GGT GGA CTT TTC ATG AGA AAC ACA GTG CAG GAG CAC AGC       207
Ile Ser Ile Gly Gly Leu Phe Met Arg Asn Thr Val Gln Glu His Ser
                10                  15                  20

GCT TTC CGC TTT GCC GTG CAG TTA TAC AAC ACC AAC CAG AAC ACC ACC       255
Ala Phe Arg Phe Ala Val Gln Leu Tyr Asn Thr Asn Gln Asn Thr Thr
            25                  30                  35

GAG AAG CCC TTC CAT TTG AAT TAC CAC GTA GAT CAC TTG GAT TCC TCC       303
Glu Lys Pro Phe His Leu Asn Tyr His Val Asp His Leu Asp Ser Ser
        40                  45                  50

AAT AGT TTT TCC GTG ACA AAT GCT TTC TGC TCC CAG TTC TCG AGA GGG       351
Asn Ser Phe Ser Val Thr Asn Ala Phe Cys Ser Gln Phe Ser Arg Gly
    55                  60                  65

GTG TAT GCC ATC TTT GGA TTC TAT GAC CAG ATG TCA ATG AAC ACC CTG       399
Val Tyr Ala Ile Phe Gly Phe Tyr Asp Gln Met Ser Met Asn Thr Leu
70                  75                  80                  85

ACC TCC TTC TGT GGG GCC CTG CAC ACA TCC TTT GTT ACG CCT AGC TTC       447
Thr Ser Phe Cys Gly Ala Leu His Thr Ser Phe Val Thr Pro Ser Phe
                90                  95                  100

CCC ACT GAC GCA GAT GTG CAG TTT GTC ATC CAG ATG CGC CCA GCC TTG       495
Pro Thr Asp Ala Asp Val Gln Phe Val Ile Gln Met Arg Pro Ala Leu
            105                 110                 115

AAG GGC GCT ATT CTG AGT CTT CTG GGT CAT TAC AAG TGG GAG AAG TTT       543
Lys Gly Ala Ile Leu Ser Leu Leu Gly His Tyr Lys Trp Glu Lys Phe
        120                 125                 130

GTG TAC CTC TAT GAC ACA GAA CGA GGA TTT TCC ATC CTC CAA GCG ATT       591
Val Tyr Leu Tyr Asp Thr Glu Arg Gly Phe Ser Ile Leu Gln Ala Ile
    135                 140                 145

ATG GAA GCA GCA GTG CAA AAC AAC TGG CAA GTA ACA GCA AGG TCT GTG       639
Met Glu Ala Ala Val Gln Asn Asn Trp Gln Val Thr Ala Arg Ser Val
150                 155                 160                 165

GGA AAC ATA AAG GAC GTC CAA GAA TTC AGG CGC ATC ATT GAA GAA ATG       687
Gly Asn Ile Lys Asp Val Gln Glu Phe Arg Arg Ile Ile Glu Glu Met
                170                 175                 180

GAC AGG AGG CAG GAA AAG CGA TAC TTG ATT GAC TGC GAA GTC GAA AGG       735
Asp Arg Arg Gln Glu Lys Arg Tyr Leu Ile Asp Cys Glu Val Glu Arg
            185                 190                 195
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ATT AAC ACA ATT TTG GAA CAG GTT GTG ATC CTA GGG AAA CAC TCA AGA      783
Ile Asn Thr Ile Leu Glu Gln Val Val Ile Leu Gly Lys His Ser Arg
        200                 205                 210

GGT TAT CAC TAC ATG CTC GCT AAC CTG GGT TTT ACT GAT ATT TTA CTG      831
Gly Tyr His Tyr Met Leu Ala Asn Leu Gly Phe Thr Asp Ile Leu Leu
    215                 220                 225

GAA AGA GTC ATG CAT GGG GGA GCC AAC ATT ACA GGT TTC CAG ATT GTC      879
Glu Arg Val Met His Gly Gly Ala Asn Ile Thr Gly Phe Gln Ile Val
230                 235                 240                 245

AAC AAT GAA AAC CCT ATG GTT CAG CAG TTC ATA CAG CGC TGG GTG AGG      927
Asn Asn Glu Asn Pro Met Val Gln Gln Phe Ile Gln Arg Trp Val Arg
                250                 255                 260

CTG GAT GAA AGG GAA TTC CCT GAA GCC AAG AAT GCA CCA CTA AAG TAT      975
Leu Asp Glu Arg Glu Phe Pro Glu Ala Lys Asn Ala Pro Leu Lys Tyr
            265                 270                 275

ACA TCT GCA TTG ACA CAC GAC GCA ATA CTG GTC ATA GCA GAA GCT TTC     1023
Thr Ser Ala Leu Thr His Asp Ala Ile Leu Val Ile Ala Glu Ala Phe
        280                 285                 290

CGC TAC CTG AGG AGG CAG CGA GTA GAT GTG TCC CGG AGA GGA AGT GCT     1071
Arg Tyr Leu Arg Arg Gln Arg Val Asp Val Ser Arg Arg Gly Ser Ala
    295                 300                 305

GGA GAC TGC TTA GCA AAT CCT GCT GTG CCC TGG AGT CAA GGA ATT GAT     1119
Gly Asp Cys Leu Ala Asn Pro Ala Val Pro Trp Ser Gln Gly Ile Asp
310                 315                 320                 325

ATT GAG AGA GCT CTG AAA ATG GTG CAA GTA CAA GGA ATG ACT GGA AAT     1167
Ile Glu Arg Ala Leu Lys Met Val Gln Val Gln Gly Met Thr Gly Asn
                330                 335                 340

ATT CAA TTT GAC ACT TAT GGA CGT AGG ACA AAT TAT ACC ATC GAT GTG     1215
Ile Gln Phe Asp Thr Tyr Gly Arg Arg Thr Asn Tyr Thr Ile Asp Val
            345                 350                 355

TAT GAA ATG AAA GTC AGT GGC TCT CGA AAA GCT GGC TAC TGG AAC GAG     1263
Tyr Glu Met Lys Val Ser Gly Ser Arg Lys Ala Gly Tyr Trp Asn Glu
        360                 365                 370

TAT GAA AGG TTT GTG CCT TTC TCA GAT CAG CAA ATC AGC AAT GAC AGT     1311
Tyr Glu Arg Phe Val Pro Phe Ser Asp Gln Gln Ile Ser Asn Asp Ser
    375                 380                 385

GCA TCC TCA GAG AAT CGG ACC ATA GTA GTG ACT ACC ATT CTG GAA TCA     1359
Ala Ser Ser Glu Asn Arg Thr Ile Val Val Thr Thr Ile Leu Glu Ser
390                 395                 400                 405

CCA TAT GTA ATG TAC AAG AAG AAC CAT GAG CAA CTG GAA GGA AAT GAA     1407
Pro Tyr Val Met Tyr Lys Lys Asn His Glu Gln Leu Glu Gly Asn Glu
                410                 415                 420

CGA TAT GAA GGC TAT TGT GTA GAC CTA GCC TAT GAA ATA GCC AAA CAT     1455
Arg Tyr Glu Gly Tyr Cys Val Asp Leu Ala Tyr Glu Ile Ala Lys His
            425                 430                 435

GTA AGG ATC AAA TAC AAA TTG TCC ATC GTT GGT GAC GGG AAA TAT GGT     1503
Val Arg Ile Lys Tyr Lys Leu Ser Ile Val Gly Asp Gly Lys Tyr Gly
        440                 445                 450

GCA AGG GAT CCA GAG ACT AAA ATA TGG AAC GGC ATG GTT GGG GAA CTT     1551
Ala Arg Asp Pro Glu Thr Lys Ile Trp Asn Gly Met Val Gly Glu Leu
    455                 460                 465
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GTC TAT GGG AGA GCT GAT ATA GCT GTT GCT CCA CTC ACT ATA ACA TTG        1599
Val Tyr Gly Arg Ala Asp Ile Ala Val Ala Pro Leu Thr Ile Thr Leu
470                 475                 480                 485

GTC CGT GAA GAA GTC ATA GAT TTT TCA AAG CCA TTA ATG AGC CTG GGC        1647
Val Arg Glu Glu Val Ile Asp Phe Ser Lys Pro Leu Met Ser Leu Gly
                490                 495                 500

ATC TCC ATC ATG ATA AAG AAG CCT CAG AAA TCA AAA CCA GGC GTA TTC        1695
Ile Ser Ile Met Ile Lys Lys Pro Gln Lys Ser Lys Pro Gly Val Phe
            505                 510                 515

TCA TTT CTG GAT CCC CTG GCT TAT GAA ATC TGG ATG TGC ATT GTC TTT        1743
Ser Phe Leu Asp Pro Leu Ala Tyr Glu Ile Trp Met Cys Ile Val Phe
        520                 525                 530

GCT TAC ATT GGA GTC AGC GTA GTT CTT TTC CTA GTC AGC AGG TTC AGT        1791
Ala Tyr Ile Gly Val Ser Val Val Leu Phe Leu Val Ser Arg Phe Ser
    535                 540                 545

CCT TAT GAA TGG CAC TTG GAA GAC AAC AAT GAA GAA CCT CGT GAC CCA        1839
Pro Tyr Glu Trp His Leu Glu Asp Asn Asn Glu Glu Pro Arg Asp Pro
550                 555                 560                 565

CAA AGT CCT CCT GAT CCT CCA AAT GAA TTT GGA ATA TTT AAC AGT CTT        1887
Gln Ser Pro Pro Asp Pro Pro Asn Glu Phe Gly Ile Phe Asn Ser Leu
                570                 575                 580

TGG TTT TCC TTG GGT GCC TTT ATG CAG CAA GGA TGT GAT ATT TCT CCA        1935
Trp Phe Ser Leu Gly Ala Phe Met Gln Gln Gly Cys Asp Ile Ser Pro
            585                 590                 595

AGA TCA CTC TCC GGG CGC ATT GTT GGA GGG GTT TGG TGG TTC TTC ACC        1983
Arg Ser Leu Ser Gly Arg Ile Val Gly Gly Val Trp Trp Phe Phe Thr
        600                 605                 610

CTG ATC ATA ATT TCT TCC TAT ACT GCC AAT CTC GCT GCT TTC CTG ACT        2031
Leu Ile Ile Ile Ser Ser Tyr Thr Ala Asn Leu Ala Ala Phe Leu Thr
    615                 620                 625

GTG GAG AGG ATG GTT TCT CCC ATA GAG AGT GCT GAA GAC TTA GCT AAA        2079
Val Glu Arg Met Val Ser Pro Ile Glu Ser Ala Glu Asp Leu Ala Lys
630                 635                 640                 645

CAG ACT GAA ATT GCA TAT GGG ACC CTG GAC TCC GGT TCA ACA AAA GAA        2127
Gln Thr Glu Ile Ala Tyr Gly Thr Leu Asp Ser Gly Ser Thr Lys Glu
                650                 655                 660

TTT TTC AGA AGA TCC AAA ATT GCT GTG TAC GAG AAA ATG TGG TCT TAC        2175
Phe Phe Arg Arg Ser Lys Ile Ala Val Tyr Glu Lys Met Trp Ser Tyr
            665                 670                 675

ATG AAA TCA GCG GAG CCA TCT GTG TTT ACC AAA ACA ACA GCA GAC GGA        2223
Met Lys Ser Ala Glu Pro Ser Val Phe Thr Lys Thr Thr Ala Asp Gly
        680                 685                 690

GTG GCC CGA GTG CGA AAG TCC AAG GGA AAG TTC GCC TTC CTG CTG GAG        2271
Val Ala Arg Val Arg Lys Ser Lys Gly Lys Phe Ala Phe Leu Leu Glu
    695                 700                 705

TCA ACC ATG AAT GAG TAC ATT GAG CAG AGA AAA CCA TGT GAT ACG ATG        2319
Ser Thr Met Asn Glu Tyr Ile Glu Gln Arg Lys Pro Cys Asp Thr Met
710                 715                 720                 725

AAA GTT GGT GGA AAT CTG GAT TCC AAA GGC TAT GGT GTG GCA ACC CCT        2367
Lys Val Gly Gly Asn Leu Asp Ser Lys Gly Tyr Gly Val Ala Thr Pro
                730                 735                 740
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AAA GGC TCA GCA TTA GGA ACG CCT GTA AAC CTT GCA GTA TTG AAA CTC 2415
Lys Gly Ser Ala Leu Gly Thr Pro Val Asn Leu Ala Val Leu Lys Leu
745 750 755

AGT GAA CAA GGC ATC TTA GAC AAG CTG AAA AAC AAA TGG TGG TAC GAT 2463
Ser Glu Gln Gly Ile Leu Asp Lys Leu Lys Asn Lys Trp Trp Tyr Asp
760 765 770

AAG GGG GAA TGT GGA GCC AAG GAC TCC GGG AGT AAG GAC AAG ACC AGC 2511
Lys Gly Glu Cys Gly Ala Lys Asp Ser Gly Ser Lys Asp Lys Thr Ser
775 780 785

GCT CTG AGC CTG AGC AAT GTG GCA GGC GTT TTC TAT ATA CTT GTC GGA 2559
Ala Leu Ser Leu Ser Asn Val Ala Gly Val Phe Tyr Ile Leu Val Gly
790 795 800 805

GGT CTG GGG CTG GCC ATG ATG GTG GCT TTG ATA GAA TTC TGT TAC AAA 2607
Gly Leu Gly Leu Ala Met Met Val Ala Leu Ile Glu Phe Cys Tyr Lys
810 815 820

TCA CGG GCA GAG TCC AAA CGC ATG AAA CTC ACA AAG AAC ACC CAA AAC 2655
Ser Arg Ala Glu Ser Lys Arg Met Lys Leu Thr Lys Asn Thr Gln Asn
825 830 835

TTT AAG CCT GCT CCT GCC ACC AAC ACT CAG AAT TAT GCT ACA TAC AGA 2703
Phe Lys Pro Ala Pro Ala Thr Asn Thr Gln Asn Tyr Ala Thr Tyr Arg
840 845 850

GAA GGC TAC AAC GTG TAT GGA ACA GAG AGT GTT AAG ATC TAGGGATCCC 2752
Glu Gly Tyr Asn Val Tyr Gly Thr Glu Ser Val Lys Ile
855 860 865

TTCCCACTGG AGGCATGTGA TGAGAGGAAA TCACCGAAAA CGTGGCTGCT TCAAGGATCC 2812

TGAGCCAGAT TTCACTCTCC TTGGTGTCGG GCATGACACG AATATTGCTG ATGGTGCAAT 2872

GACCTTTCAA TAGGAAAAAC TGATTTTTTT TTTCCTTCAG TGCCTTATGG AACACTCTGA 2932

GACTCGCGAC AATGCAAACC ATCATTGAAA TCTTTTTGCT TTGCTTGAAA AAAAATAATT 2992

AAAATAAAAA CCAACAAAAA TGGACATGCA TCAAACCCTT GATGTATTAA TATTTATTAT 3052

AGTTTTCATT AGGAATTC 3070

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 888 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Met Gly Gln Ser Val Leu Arg Ala Val Phe Phe Leu Val Leu Gly Leu
-22 -20 -15 -10

Leu Gly His Ser His Gly Gly Phe Pro Asn Thr Ile Ser Ile Gly Gly
-5 1 5 10

Leu Phe Met Arg Asn Thr Val Gln Glu His Ser Ala Phe Arg Phe Ala
15 20 25

Val Gln Leu Tyr Asn Thr Asn Gln Asn Thr Thr Glu Lys Pro Phe His
30 35 40

```
Leu Asn Tyr His Val Asp His Leu Asp Ser Ser Asn Ser Phe Ser Val
            45                  50                  55

Thr Asn Ala Phe Cys Ser Gln Phe Ser Arg Gly Val Tyr Ala Il  Phe
        60                  65                  70

Gly Phe Tyr Asp Gln Met Ser Met Asn Thr Leu Thr Ser Phe Cys Gly
 75                  80                  85                  90

Ala Leu His Thr Ser Phe Val Thr Pro Ser Phe Pro Thr Asp Ala Asp
                 95                 100                 105

Val Gln Phe Val Ile Gln Met Arg Pro Ala Leu Lys Gly Ala Ile Leu
            110                 115                 120

Ser Leu Leu Gly His Tyr Lys Trp Glu Lys Phe Val Tyr Leu Tyr Asp
        125                 130                 135

Thr Glu Arg Gly Phe Ser Ile Leu Gln Ala Ile Met Glu Ala Ala Val
    140                 145                 150

Gln Asn Asn Trp Gln Val Thr Ala Arg Ser Val Gly Asn Ile Lys Asp
155                 160                 165                 170

Val Gln Glu Phe Arg Arg Ile Ile Glu Glu Met Asp Arg Arg Gln Glu
                175                 180                 185

Lys Arg Tyr Leu Ile Asp Cys Glu Val Glu Arg Ile Asn Thr Ile Leu
            190                 195                 200

Glu Gln Val Val Ile Leu Gly Lys His Ser Arg Gly Tyr His Tyr Met
        205                 210                 215

Leu Ala Asn Leu Gly Phe Thr Asp Ile Leu Leu Glu Arg Val Met His
    220                 225                 230

Gly Gly Ala Asn Ile Thr Gly Phe Gln Ile Val Asn Asn Glu Asn Pro
235                 240                 245                 250

Met Val Gln Gln Phe Ile Gln Arg Trp Val Arg Leu Asp Glu Arg Glu
                255                 260                 265

Phe Pro Glu Ala Lys Asn Ala Pro Leu Lys Tyr Thr Ser Ala Leu Thr
            270                 275                 280

His Asp Ala Ile Leu Val Ile Ala Glu Ala Phe Arg Tyr Leu Arg Arg
        285                 290                 295

Gln Arg Val Asp Val Ser Arg Arg Gly Ser Ala Gly Asp Cys Leu Ala
    300                 305                 310

Asn Pro Ala Val Pro Trp Ser Gln Gly Ile Asp Ile Glu Arg Ala Leu
315                 320                 325                 330

Lys Met Val Gln Val Gln Gly Met Thr Gly Asn Ile Gln Phe Asp Thr
                335                 340                 345

Tyr Gly Arg Arg Thr Asn Tyr Thr Ile Asp Val Tyr Glu Met Lys Val
            350                 355                 360

Ser Gly Ser Arg Lys Ala Gly Tyr Trp Asn Glu Tyr Glu Arg Phe Val
        365                 370                 375

Pro Phe Ser Asp Gln Gln Ile Ser Asn Asp Ser Ala Ser Ser Glu Asn
    380                 385                 390
```

Arg Thr Ile Val Val Thr Thr Ile Leu Glu Ser Pro Tyr Val Met Tyr
395 400 405 410

Lys Lys Asn His Glu Gln Leu Glu Gly Asn Glu Arg Tyr Glu Gly Tyr
415 420 425

Cys Val Asp Leu Ala Tyr Glu Ile Ala Lys His Val Arg Ile Lys Tyr
430 435 440

Lys Leu Ser Ile Val Gly Asp Gly Lys Tyr Gly Ala Arg Asp Pro Glu
445 450 455

Thr Lys Ile Trp Asn Gly Met Val Gly Glu Leu Val Tyr Gly Arg Ala
460 465 470

Asp Ile Ala Val Ala Pro Leu Thr Ile Thr Leu Val Arg Glu Glu Val
475 480 485 490

Ile Asp Phe Ser Lys Pro Leu Met Ser Leu Gly Ile Ser Ile Met Ile
495 500 505

Lys Lys Pro Gln Lys Ser Lys Pro Gly Val Phe Ser Phe Leu Asp Pro
510 515 520

Leu Ala Tyr Glu Ile Trp Met Cys Ile Val Phe Ala Tyr Ile Gly Val
525 530 535

Ser Val Val Leu Phe Leu Val Ser Arg Phe Ser Pro Tyr Glu Trp His
540 545 550

Leu Glu Asp Asn Asn Glu Glu Pro Arg Asp Pro Gln Ser Pro Pro Asp
555 560 565 570

Pro Pro Asn Glu Phe Gly Ile Phe Asn Ser Leu Trp Phe Ser Leu Gly
575 580 585

Ala Phe Met Gln Gln Gly Cys Asp Ile Ser Pro Arg Ser Leu Ser Gly
590 595 600

Arg Ile Val Gly Gly Val Trp Trp Phe Phe Thr Leu Ile Ile Ile Ser
605 610 615

Ser Tyr Thr Ala Asn Leu Ala Ala Phe Leu Thr Val Glu Arg Met Val
620 625 630

Ser Pro Ile Glu Ser Ala Glu Asp Leu Ala Lys Gln Thr Glu Ile Ala
635 640 645 650

Tyr Gly Thr Leu Asp Ser Gly Ser Thr Lys Glu Phe Phe Arg Arg Ser
655 660 665

Lys Ile Ala Val Tyr Glu Lys Met Trp Ser Tyr Met Lys Ser Ala Glu
670 675 680

Pro Ser Val Phe Thr Lys Thr Thr Ala Asp Gly Val Ala Arg Val Arg
685 690 695

Lys Ser Lys Gly Lys Phe Ala Phe Leu Leu Glu Ser Thr Met Asn Glu
700 705 710

Tyr Ile Glu Gln Arg Lys Pro Cys Asp Thr Met Lys Val Gly Gly Asn
715 720 725 730

Leu Asp Ser Lys Gly Tyr Gly Val Ala Thr Pro Lys Gly Ser Ala Leu
735 740 745

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Gly Thr Pro Val Asn Leu Ala Val Leu Lys Leu Ser Glu Gln Gly Ile
            750                 755                 760

Leu Asp Lys Leu Lys Asn Lys Trp Trp Tyr Asp Lys Gly Glu Cys Gly
        765                 770                 775

Ala Lys Asp Ser Gly Ser Lys Asp Lys Thr Ser Ala Leu Ser Leu Ser
    780                 785                 790

Asn Val Ala Gly Val Phe Tyr Ile Leu Val Gly Gly Leu Gly Leu Ala
795                 800                 805                     810

Met Met Val Ala Leu Ile Glu Phe Cys Tyr Lys Ser Arg Ala Glu Ser
                815                 820                 825

Lys Arg Met Lys Leu Thr Lys Asn Thr Gln Asn Phe Lys Pro Ala Pro
            830                 835                 840

Ala Thr Asn Thr Gln Asn Tyr Ala Thr Tyr Arg Glu Gly Tyr Asn Val
        845                 850                 855

Tyr Gly Thr Glu Ser Val Lys Ile
    860                 865
```

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 46 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

```
Gly Ser Ala Leu Gly Asn Ala Val Asn Leu Ala Val Leu Lys Leu Asn
1               5                   10                  15

Glu Gln Gly Leu Leu Asp Lys Leu Lys Asn Lys Trp Trp Tyr Asp Lys
            20                  25                  30

Gly Glu Cys Gly Ser Gly Gly Gly Asp Ser Lys Asp Lys Thr
        35                  40                  45
```

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 46 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

```
Gly Ser Ala Leu Gly Thr Pro Val Asn Leu Ala Val Leu Lys Leu Ser
1               5                   10                  15

Glu Gln Gly Ile Leu Asp Lys Leu Lys Asn Lys Trp Trp Tyr Asp Lys
            20                  25                  30
```

Gly Glu Cys Gly Ala Lys Asp Ser Gly Ser Lys Asp Lys Thr
35 40 45

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Other nucleic acid;
(A) DESCRIPTION: Synthetic DNA oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

AGCTTGCGGC CGC 13

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Other nucleic acid;
(A) DESCRIPTION: Synthetic DNA oligonucleotide

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

GCGGCCGCA 9

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 40 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Other nucleic acid;
(A) DESCRIPTION: Synthetic DNA oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

ACACTCAGAA TTACGCTACA TACAGAGAAG GCTACAACGT 40

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 40 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Other nucleic acid;
(A) DESCRIPTION: Synthetic DNA oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

CCAGATCGAT ATTGTGAACA TCAGCGACAC GTTTGAGATG 40

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 32 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Other nucleic acid;
(A) DESCRIPTION: Synthetic DNA oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

GTGAATGTGG AGCCAAGGAC TCGGGAAGTA AG 32

Claims

1. An isolated polynucleotide comprising a region which encodes an AMPA-binding human GluR receptor selected from the group consisting of human GluR1B, GluR2B, GluR3A and GluR3B receptors, and AMPA-binding fragments thereof.

2. An isolated polynucleotide according to claim 1, which encodes said GluR1B receptor, said GluR2B receptor, said GluR3A receptor or said GluR3B receptor.

3. An isolated polynucleotide comprising a region which encodes an AMPA-binding variant of a GluR receptor selected from the group consisting of human GluR1B, GluR2B, GluR3A and GluR3B receptors, wherein said variant has the binding profile of said receptor and varies from said receptor by conservative amino acid substitution.

4. An isolated polynucleotide according to any one of claims 1 to 3, which consists of DNA.

5. A recombinant DNA construct having incorporated therein a polynucleotide as defined in any one of claims 1 to 4.

6. A recombinant DNA construct according to claim 5, wherein the polynucleotide incorporated therein is linked operably with DNA enabling expression and secretion of said receptor in a cellular host.

7. A recombinant DNA construct according to claim 5, which is plasmid pBS/humGluR3A (ATCC 75218), plasmid pBS/humGluR3B (ATCC 75219); plasmid pBS/humGluR1B (ATCC 75246) or plasmid pBS/humGluR2B (ATCC 75217).

8. A cellular host having incorporated therein a heterologous polynucleotide as defined in any one of claims 1 to 4.

9. A cellular host according to claim 8, which is a mammalian cell.

10. An AMPA-binding membrane preparation derived from a cellular host as defined in claim 8 or claim 9.

11. A process for obtaining a substantially homogeneous source of human GluR receptor, which comprises the step of culturing a cellular host as defined in claim 8 or claim 9, and then recovering the cells so cultured.

12. A process for obtaining a substantially homogeneous source of human GluR receptor according to claim

11 comprising the subsequent step of obtaining a membrane preparation from the cultured cells.

13. A method of assaying a substance for binding to a human EAA receptor, which comprises the steps of incubating the substance under appropriate conditions with a cellular host as defined in claim 8 or claim 9, or with an AMPA-binding membrane preparation derived therefrom, and determining the extent of binding between the human GluR receptor and the substance.

14. An isolated human GluR receptor selected from the group consisting of GluR1B, GluR2B, GluR3A and GluR3B receptors, and AMPA-binding fragments thereof, in a form essentially free from other proteins of human origin.

15. An AMPA-binding fragment of a human GluR receptor selected from the group consisting of GluR1B, GluR2B, GluR3A and GluR3A receptors.

16. An antibody which binds a human GluR receptor selected from the group consisting of GluR1B, GluR2B, GluR3A and GluR3B receptors.

17. An immunogenic fragment of a human GluR receptor selected from the group consisting of GluR1B, GluR2B, GluR3A and GluR3B receptors.

18. An oligonucleotide which comprises at least about 17 nucleic acids and which hybridizes selectively with a polynucleotide defined in any one of claims 1 to 4.

FIG. 1A

```
     EcoRI
     |
     GAATTCCACACCAAATCTATGATTGGACCTGGGCTTCTTTTTCGCCAATGCAAAAAGGAA
   1 ---------+---------+---------+---------+---------+---------+ 60
     CTTAAGGTGTGGTTTAGATACTAACCTGGACCCGAAGAAAAAGCGGTTACGTTTTTCCTT

     TATGCAGCACATTTTTGCCTTCTTCTGCACCGGTTTCCTAGGCGCGGTAGTAGGTGCCAA
  61 ---------+---------+---------+---------+---------+---------+ 120
     ATACGTCGTGTAAAAACGGAAGAAGACGTGGCCAAAGGATCCGCGCCATCATCCACGGTT

      M  Q  H  I  F  A  F  F  C  T  G  F  L  G  A  V  V  G  A| N  -

     TTTCCCCAACAATATCCAGATCGGGGGATTATTTCCAAACCAGCAGTCACAGGAACATGC
 121 ---------+---------+---------+---------+---------+---------+ 180
     AAAGGGGTTGTTATAGGTCTAGCCCCCTAATAAAGGTTTGGTCGTCAGTGTCCTTGTACG

      F  P  N  N  I  Q  I  G  G  L  F  P  N  Q  Q  S  Q  E  H  A  -

     TGCTTTTAGATTTGCTTTGTCGCAACTCACAGAGCCCCCGAAGCTGCTCCCCCAGATTGA
 181 ---------+---------+---------+---------+---------+---------+ 240
     ACGAAAATCTAAACGAAACAGCGTTGAGTGTCTCGGGGGCTTCGACGAGGGGGTCTAACT

      A  F  R  F  A  L  S  Q  L  T  E  P  P  K  L  L  P  Q  I  D  -

     TATTGTGAACATCAGCGACACGTTTGAGATGACCTATAGATTCTGTTCCCAGTTCTCCAA
 241 ---------+---------+---------+---------+---------+---------+ 300
     ATAACACTTGTAGTCGCTGTGCAAACTCTACTGGATATCTAAGACAAGGGTCAAGAGGTT

      I  V  N  I  S  D  T  F  E  M  T  Y  R  F  C  S  Q  F  S  K  -
```

FIG. 1B

```
     AGGAGTCTATGCCATCTTTGGGTTTTATGAACGTAGGACTGTCAACATGCTGACCTCCTT
301  ---------+---------+---------+---------+---------+---------+ 360
     TCCTCAGATACGGTAGAAACCCAAAATACTTGCATCCTGACAGTTGTACGACTGGAGGAA

      G  V  Y  A  I  F  G  F  Y  E  R  R  T  V  N  M  L  T  S  F  -

     TTGTGGGGCCCTCCACGTCTGCTTCATTACGCCGAGCTTTCCCGTTGATACATCCAATCA
361  ---------+---------+---------+---------+---------+---------+ 420
     AACACCCCGGGAGGTGCAGACGAAGTAATGCGGCTCGAAAGGGCAACTATGTAGGTTAGT

      C  G  A  L  H  V  C  F  I  T  P  S  F  P  V  D  T  S  N  Q  -

     GTTTGTCCTTCAGCTGCGCCCTGAACTGCAGGATGCCCTCATCAGCATCATTGACCATTA
421  ---------+---------+---------+---------+---------+---------+ 480
     CAAACAGGAAGTCGACGCGGGACTTGACGTCCTACGGGAGTAGTCGTAGTAACTGGTAAT

      F  V  L  Q  L  R  P  E  L  Q  D  A  L  I  S  I  I  D  H  Y  -

     CAAGTGGCAGAAATTTGTCTACATTTATGATGCCGACCGGGGCTTATCCGTCCTGCAGAA
481  ---------+---------+---------+---------+---------+---------+ 540
     GTTCACCGTCTTTAAACAGATGTAAATACTACGGCTGGCCCCGAATAGGCAGGACGTCTT

      K  W  Q  K  F  V  Y  I  Y  D  A  D  R  G  L  S  V  L  Q  K  -

     AGTCCTGGATACAGCTGCTGAGAAGAACTGGCAGGTGACAGCAGTCAACATTTTGACAAC
541  ---------+---------+---------+---------+---------+---------+ 600
     TCAGGACCTATGTCGACGACTCTTCTTGACCGTCCACTGTCGTCAGTTGTAAAACTGTTG

      V  L  D  T  A  A  E  K  N  W  Q  V  T  A  V  N  I  L  T  T  -
```

FIG. 1C

```
    CACAGAGGAGGGATACCGGATGCTCTTTCAGGACCTGGAGAAGAAAAAGGAGCGGCTGGT
601 ---------+---------+---------+---------+---------+---------+ 660
    GTGTCTCCTCCCTATGGCCTACGAGAAAGTCCTGGACCTCTTCTTTTTCCTCGCCGACCA

     T  E  E  G  Y  R  M  L  F  Q  D  L  E  K  K  K  E  R  L  V  -

    GGTGGTGGACTGTGAATCAGAACGCCTCAATGCTATCTTGGGCCAGATTATAAAGCTAGA
661 ---------+---------+---------+---------+---------+---------+ 720
    CCACCACCTGACACTTAGTCTTGCGGAGTTACGATAGAACCCGGTCTAATATTTCGATCT

     V  V  D  C  E  S  E  R  L  N  A  I  L  G  Q  I  I  K  L  E  -

    GAAGAATGGCATCGGCTACCACTACATTCTTGCAAATCTGGGCTTCATGGACATTGACTT
721 ---------+---------+---------+---------+---------+---------+ 780
    CTTCTTACCGTAGCCGATGGTGATGTAAGAACGTTTAGACCCGAAGTACCTGTAACTGAA

     K  N  G  I  G  Y  H  Y  I  L  A  N  L  G  F  M  D  I  D  L  -

    AAACAAATTCAAGGAGAGTGGCGCCAATGTGACAGGTTTCCAGCTGGTGAACTACACAGA
781 ---------+---------+---------+---------+---------+---------+ 840
    TTTGTTTAAGTTCCTCTCACCGCGGTTACACTGTCCAAAGGTCGACCACTTGATGTGTCT

     N  K  F  K  E  S  G  A  N  V  T  G  F  Q  L  V  N  Y  T  D  -

    CACTATTCCGGCCAAGATCATGCAGCAGTGGAAGAATAGTGATGCTCGAGACCACACACG
841 ---------+---------+---------+---------+---------+---------+ 900
    GTGATAAGGCCGGTTCTAGTACGTCGTCACCTTCTTATCACTACGAGCTCTGGTGTGTGC

     T  I  P  A  K  I  M  Q  Q  W  K  N  S  D  A  R  D  H  T  R  -
```

FIG. 1D

```
      GGTGGACTGGAAGAGACCCAAGTACACCTCTGCGCTCACCTACGATGGGGTGAAGGTGAT
 901 ---------+---------+---------+---------+---------+---------+ 960
      CCACCTGACCTTCTCTGGGTTCATGTGGAGACGCGAGTGGATGCTACCCCACTTCCACTA

       V  D  W  K  R  P  K  Y  T  S  A  L  T  Y  D  G  V  K  V  M  -

      GGCTGAGGCTTTCCAGAGCCTGCGGAGGCAGAGAATTGATATATCTCGCCGGGGGAATGC
 961 ---------+---------+---------+---------+---------+---------+ 1020
      CCGACTCCGAAAGGTCTCGGACGCCTCCGTCTCTTAACTATATAGAGCGGCCCCCTTACG

       A  E  A  F  Q  S  L  R  R  Q  R  I  D  I  S  R  R  G  N  A  -

      TGGGGATTGTCTGGCTAACCCAGCTGTTCCCTGGGGCCAAGGGATCGACATCCAGAGAGC
1021 ---------+---------+---------+---------+---------+---------+ 1080
      ACCCCTAACAGACCGATTGGGTCGACAAGGGACCCCGGTTCCCTAGCTGTAGGTCTCTCG

       G  D  C  L  A  N  P  A  V  P  W  G  Q  G  I  D  I  Q  R  A  -

      TCTGCAGCAGGTGCGATTTGAAGGTTTAACAGGAAACGTGCAGTTTAATGAGAAAGGACG
1081 ---------+---------+---------+---------+---------+---------+ 1140
      AGACGTCGTCCACGCTAAACTTCCAAATTGTCCTTTGCACGTCAAATTACTCTTTCCTGC

       L  Q  Q  V  R  F  E  G  L  T  G  N  V  Q  F  N  E  K  G  R  -

      CCGGACCAACTACACGCTCCACGTGATTGAAATGAAACATGACGGCATCCGAAAGATTGG
1141 ---------+---------+---------+---------+---------+---------+ 1200
      GGCCTGGTTGATGTGCGAGGTGCACTAACTTTACTTTGTACTGCCGTAGGCTTTCTAACC

       R  T  N  Y  T  L  H  V  I  E  M  K  H  D  G  I  R  K  I  G  -
```

FIG. 1E

```
     TTACTGGAATGAAGATGATAAGTTTGTCCCTGCAGCCACCGATGCCCAAGCTGGGGGCGA
1201 ---------+---------+---------+---------+---------+---------+ 1260
     AATGACCTTACTTCTACTATTCAAACAGGGACGTCGGTGGCTACGGGTTCGACCCCCGCT

      Y  W  N  E  D  D  K  F  V  P  A  A  T  D  A  Q  A  G  G  D  -

     TAATTCAAGTGTTCAGAACAGAACATACATCGTCACAACAATCCTAGAAGATCCTTATGT
1261 ---------+---------+---------+---------+---------+---------+ 1320
     ATTAAGTTCACAAGTCTTGTCTTGTATGTAGCAGTGTTGTTAGGATCTTCTAGGAATACA

      N  S  S  V  Q  N  R  T  Y  I  V  T  T  I  L  E  D  P  Y  V  -

     GATGCTCAAGAAGAACGCCAATCAGTTTGAGGGCAATGACCGTTACGAGGGCTACTGTGT
1321 ---------+---------+---------+---------+---------+---------+ 1380
     CTACGAGTTCTTCTTGCGGTTAGTCAAACTCCCGTTACTGGCAATGCTCCCGATGACACA

      M  L  K  K  N  A  N  Q  F  E  G  N  D  R  Y  E  G  Y  C  V  -

     AGAGCTGGCGGCAGAGATTGCCAAGCACGTGGGCTACTCCTACCGTCTGGAGATTGTCAG
1381 ---------+---------+---------+---------+---------+---------+ 1440
     TCTCGACCGCCGTCTCTAACGGTTCGTGCACCCGATGAGGATGGCAGACCTCTAACAGTC

      E  L  A  A  E  I  A  K  H  V  G  Y  S  Y  R  L  E  I  V  S  -

     TGATGGAAAATACGGAGCCCGAGACCCTGACACGAAGGCCTGGAATGGCATGGTGGGAGA
1441 ---------+---------+---------+---------+---------+---------+ 1500
     ACTACCTTTTATGCCTCGGGCTCTGGGACTGTGCTTCCGGACCTTACCGTACCACCCTCT

      D  G  K  Y  G  A  R  D  P  D  T  K  A  W  N  G  M  V  G  E  -
```

FIG. 1F

```
     GCTGGTCTATGGAAGAGCAGATGTGGCTGTGGCTCCCTTAACTATCACTTTGGTCCGGGA
1501 ---------+---------+---------+---------+---------+---------+ 1560
     CGACCAGATACCTTCTCGTCTACACCGACACCGAGGGAATTGATAGTGAAACCAGGCCCT

      L  V  Y  G  R  A  D  V  A  V  A  P  L  T  I  T  L  V  R  E  -

     AGAAGTTATAGATTTCTCCAAACCATTTATGAGTTTGGGGATCTCCATCATGATTAAAAA
1561 ---------+---------+---------+---------+---------+---------+ 1620
     TCTTCAATATCTAAAGAGGTTTGGTAAATACTCAAACCCCTAGAGGTAGTACTAATTTTT

      E  V  I  D  F  S  K  P  F  M  S  L  G  I  S  I  M  I  K  K  -

     ACCACAGAAATCCAAGCCGGGTGTCTTCTCCTTCCTTGATCCTTTGGCTTATGAGATTTG
1621 ---------+---------+---------+---------+---------+---------+ 1680
     TGGTGTCTTTAGGTTCGGCCCACAGAAGAGGAAGGAACTAGGAAACCGAATACTCTAAAC

      P  Q  K  S  K  P  G  V  F  S  F  L  D  P  L  A  Y  E  I  W  -

     GATGTGCATTGTTTTTGCCTACATTGGAGTGAGTGTTGTCCTCTTCCTGGTCAGCCGCTT
1681 ---------+---------+---------+---------+---------+---------+ 1740
     CTACACGTAACAAAAACGGATGTAACCTCACTCACAACAGGAGAAGGACCAGTCGGCGAA

      M  C  I  V  F  A  Y  I  G  V  S  V  V  L  F  L  V  S  R  F  -

     CAGTCCCTATGAATGGCACAGTGAAGAGTTTGAGGAAGGACGGGACCAGACAACCAGTGA
1741 ---------+---------+---------+---------+---------+---------+ 1800
     GTCAGGGATACTTACCGTGTCACTTCTCAAACTCCTTCCTGCCCTGGTCTGTTGGTCACT

      S  P  Y  E  W  H  S  E  E  F  E  E  G  R  D  Q  T  T  S  D  -
```

FIG. 1G

```
     CCAGTCCAATGAGTTTGGGATATTCAACAGTTTGTGGTTCTCCCTGGGAGCCTTCATGCA
1801 ---------+---------+---------+---------+---------+---------+ 1860
     GGTCAGGTTACTCAAACCCTATAAGTTGTCAAACACCAAGAGGGACCCTCGGAAGTACGT

      Q  S  N  E  F  G  I  F  N  S  L  W  F  S  L  G  A  F  M  Q  -

     GCAAGGATGTGACATTTCTCCCAGGTCCCTGTCTGGTCGCATCGTTGGTGGCGTCTGGTG
1861 ---------+---------+---------+---------+---------+---------+ 1920
     CGTTCCTACACTGTAAAGAGGGTCCAGGGACAGACCAGCGTAGCAACCACCGCAGACCAC

      Q  G  C  D  I  S  P  R  S  L  S  G  R  I  V  G  G  V  W  W  -

     GTTCTTCACCTTAATCATCATCTCCTCATATACAGCCAATCTGGCCGCCTTCCTGACCGT
1921 ---------+---------+---------+---------+---------+---------+ 1980
     CAAGAAGTGGAATTAGTAGTAGAGGAGTATATGTCGGTTAGACCGGCGGAAGGACTGGCA

      F  F  T  L  I  I  I  S  S  Y  T  A  N  L  A  A  F  L  T  V  -

     GGAGAGGATGGTGTCTCCCATTGAGAGTGCAGAGGACCTAGCGAACGAGACAGAAATTGC
1981 ---------+---------+---------+---------+---------+---------+ 2040
     CCTCTCCTACCACAGAGGGTAACTCTCACGTCTCCTGGATCGCTTGCTCTGTCTTTAACG

      E  R  M  V  S  P  I  E  S  A  E  D  L  A  N  E  T  E  I  A  -

     CTACGGGACGCTGGAAGCAGGATCTACTAAGGAGTTCTTCAGGAGGTCTAAAATTGCTGT
2041 ---------+---------+---------+---------+---------+---------+ 2100
     GATGCCCTGCGACCTTCGTCCTAGATGATTCCTCAAGAAGTCCTCCAGATTTTAACGACA

      Y  G  T  L  E  A  G  S  T  K  E  F  F  R  R  S  K  I  A  V  -
```

FIG. 1H

```
     GTTTGAGAAGATGTGGACATACATGAAGTCAGCAGAGCCATCAGTTTTTGTGCGGACCAC
2101 ---------+---------+---------+---------+---------+---------+ 2160
     CAAACTCTTCTACACCTGTATGTACTTCAGTCGTCTCGGTAGTCAAAAACACGCCTGGTG

      F  E  K  M  W  T  Y  M  K  S  A  E  P  S  V  F  V  R  T  T  -

     AGAGGAGGGGATGATTCGAGTGAGGAAATCCAAAGGCAAATATGCCTACCTCCTGGAGTC
2161 ---------+---------+---------+---------+---------+---------+ 2220
     TCTCCTCCCCTACTAAGCTCACTCCTTTAGGTTTCCGTTTATACGGATGGAGGACCTCAG

      E  E  G  M  I  R  V  R  K  S  K  G  K  Y  A  Y  L  L  E  S  -

     CACCATGAATGAGTACATTGAGCAGCGGAAACCCTGTGACACCATGAAGGTGGGAGGTAA
2221 ---------+---------+---------+---------+---------+---------+ 2280
     GTGGTACTTACTCATGTAACTCGTCGCCTTTGGGACACTGTGGTACTTCCACCCTCCATT

      T  M  N  E  Y  I  E  Q  R  K  P  C  D  T  M  K  V  G  G  N  -

     CTTGGATTCCAAAGGCTATGGCATTGCAACACCCAAGGGGTCTGCCCTGAGAGGTCCCGT
2281 ---------+---------+---------+---------+---------+---------+ 2340
     GAACCTAAGGTTTCCGATACCGTAACGTTGTGGGTTCCCCAGACGGGACTCTCCAGGGCA

      L  D  S  K  G  Y  G  I  A  T  P  K  G  S  A  L  R  G  P  V  -

     AAACCTAGCGGTTTTGAAACTCAGTGAGCAAGGCGTCTTAGACAAGCTGAAAAGCAAATG
2341 ---------+---------+---------+---------+---------+---------+ 2400
     TTTGGATCGCCAAAACTTTGAGTCACTCGTTCCGCAGAATCTGTTCGACTTTTCGTTTAC

      N  L  A  V  L  K  L  S  E  Q  G  V  L  D  K  L  K  S  K  W  -
```

FIG. 11

```
     GTGGTACGATAAAGGGGAATGTGGAAGCAAGGACTCCGGAAGTAAGGACAAGACAAGCGC
2401 ---------+---------+---------+---------+---------+---------+ 2460
     CACCATGCTATTTCCCCTTACACCTTCGTTCCTGAGGCCTTCATTCCTGTTCTGTTCGCG

      W  Y  D  K  G  E  C  G  S  K  D  S  G  S  K  D  K  T  S  A  -

     TCTGAGCCTCAGCAATGTGGCAGGCGTGTTCTACATCCTGATCGGAGGACTTGGACTAGC
2461 ---------+---------+---------+---------+---------+---------+ 2520
     AGACTCGGAGTCGTTACACCGTCCGCACAAGATGTAGGACTAGCCTCCTGAACCTGATCG

      L  S  L  S  N  V  A  G  V  F  Y  I  L  I  G  G  L  G  L  A  -

     CATGCTGGTTGCCTTAATCGAGTTCTGCTACAAATCCCGTAGTGAATCCAAGCGGATGAA
2521 ---------+---------+---------+---------+---------+---------+ 2580
     GTACGACCAACGGAATTAGCTCAAGACGATGTTTAGGGCATCACTTAGGTTCGCCTACTT

      M  L  V  A  L  I  E  F  C  Y  K  S  R  S  E  S  K  R  M  K  -

     GGGTTTTTGTTTGATCCCACAGCAATCCATCAACGAAGCCATACGGACATCGACCCTCCC
2581 ---------+---------+---------+---------+---------+---------+ 2640
     CCCAAAAACAAACTAGGGTGTCGTTAGGTAGTTGCTTCGGTATGCCTGTAGCTGGGAGGG

      G  F  C  L  I  P  Q  Q  S  I  N  E  A  I  R  T  S  T  L  P  -

     CCGCAACAGCGGGGCAGGAGCCAGCAGCGGCGGCAGTGGAGAGAATGGTCGGGTGGTCAG
2641 ---------+---------+---------+---------+---------+---------+ 2700
     GGCGTTGTCGCCCCGTCCTCGGTCGTCGCCGCCGTCACCTCTCTTACCAGCCCACCAGTC

      R  N  S  G  A  G  A  S  S  G  G  S  G  E  N  G  R  V  V  S  -
```

FIG. 1J

```
        CCATGACTTCCCCAAGTCCATGCAATCGATTCCTTGCATGAGCCACAGTTCAGGGATGCC
   2701 ---------+---------+---------+---------+---------+---------+ 2760
        GGTACTGAAGGGGTTCAGGTACGTTAGCTAAGGAACGTACTCGGTGTCAAGTCCCTACGG

         H  D  F  P  K  S  M  Q  S  I  P  C  M  S  H  S  S  G  M  P  -

        CTTGGGAGCCACGGGATTGTAACTGGAGCAGATGGAGACCCCTTGGGGAGCAGGCTCGGG
   2761 ---------+---------+---------+---------+---------+---------+ 2820
        GAACCCTCGGTGCCCTAACATTGACCTCGTCTACCTCTGGGGAACCCCTCGTCCGAGCCC

         L  G  A  T  G  L  *

        CTCCCCAGCCCCATCCCAAACCCTTCAGTGCCAAAAACAACAACAAAATAGAAAGCGCAA
   2821 ---------+---------+---------+---------+---------+---------+ 2880
        GAGGGGTCGGGGTAGGGTTTGGGAAGTCACGGTTTTTGTTGTTGTTTTATCTTTCGCGTT

        CCACCACCAACCACTGCGACCACAAGAAGGATGATTCAACAGGTTTTCCTGAAGAATTGA
   2881 ---------+---------+---------+---------+---------+---------+ 2940
        GGTGGTGGTTGGTGACGCTGGTGTTCTTCCTACTAAGTTGTCCAAAAGGACTTCTTAACT

        AAAACCATTTTGCTGTCCCTTTTCCTTTTTTGATGTTCTTTCACCCTTTTCTGTTTGCTA
   2941 ---------+---------+---------+---------+---------+---------+ 3000
        TTTTGGTAAAACGACAGGGAAAAGGAAAAAACTACAAGAAAGTGGGAAAAGACAAACGAT

        AGTGAGGATGAAAAAATAACACTGTACTGCAATAAGGGGAGAGTAACCCTGTCTAATGAA
   3001 ---------+---------+---------+---------+---------+---------+ 3060
        TCACTCCTACTTTTTTATTGTGACATGACGTTATTCCCCTCTCATTGGGACAGATTACTT
```

FIG. 1K

```
     ACCTGTGTCTCTGAGAGTAGAGTCACTGGAACACTAATGAGGAAACTGCACTGTTTTATT
3061 ---------+---------+---------+---------+---------+---------+ 3120
     TGGACACAGAGACTCTCATCTCAGTGACCTTGTGATTACTCCTTTGACGTGACAAAATAA

     TTAATTCAGTTGTTAGTGTGTCTTAGTGTGTGCAATTTTTTTCTTACTAATATCCATGG
3121 ---------+---------+---------+---------+---------+---------+ 3180
     AATTAAGTCAACAATCACACAGAATCACACACGTTAAAAAAAGAATGATTATAGGTACC

                                   EcoRI
                                   |
     TTTGCAGGTTCTGTTAGGCCCTTTCCTTCTCCTGGAATTC
3181 ---------+---------+---------+---------+ 3220
     AAACGTCCAAGACAATCCGGGAAAGGAAGAGGACCTTAAG
```

FIG. 2A

```
    EcoRI
    |
    GAATTCCGTGAGTGCATGGGAGGGTGCTGAATATTCCGAGACACTGGGACCACAGCGGCA
  1 ---------+---------+---------+---------+---------+---------+ 60
    CTTAAGGCACTCACGTACCCTCCCACGACTTATAAGGCTCTGTGACCCTGGTGTCGCCGT

    GCTCCGCTGAAAACTGCATTCAGCCAGTCCTCCGGACTTCTGGAGCGGGGACAGGGCGCA
 61 ---------+---------+---------+---------+---------+---------+ 120
    CGAGGCGACTTTTGACGTAAGTCGGTCAGGAGGCCTGAAGACCTCGCCCCTGTCCCGCGT

    GGGCATCAGCAGCCACCAGCAGGACCTGGGAAATAGGGATTCTTCTGCCTCCACTTCAGG
121 ---------+---------+---------+---------+---------+---------+ 180
    CCCGTAGTCGTCGGTGGTCGTCCTGGACCCTTTATCCCTAAGAAGACGGAGGTGAAGTCC

    TTTTAGCAGCTTGGTGCTAAATTGCTGTCTCAAAATGCAGAGGATCTAATTTGCAGAGGA
181 ---------+---------+---------+---------+---------+---------+ 240
    AAAATCGTCGAACCACGATTTAACGACAGAGTTTTACGTCTCCTAGATTAAACGTCTCCT

    AAACAGCCAAAGAAGGAAGAGGAGGAAAAGGAAAAAAAAAGGGGTATATTGTGGATGCTC
241 ---------+---------+---------+---------+---------+---------+ 300
    TTTGTCGGTTTCTTCCTTCTCCTCCTTTTCCTTTTTTTTTCCCCATATAACACCTACGAG

    TACTTTTCTTGGAAATGCAAAAGATTATGCATATTTCTGTCCTCCTTTCTCCTGTTTTAT
301 ---------+---------+---------+---------+---------+---------+ 360
    ATGAAAAGAACCTTTACGTTTTCTAATACGTATAAAGACAGGAGGAAAGAGGACAAAATA

                  M  Q  K  I  M  H  I  S  V  L  L  S  P  V  L  W -

    GGGGACTGATTTTTGGTGTCTCTTCTAACAGCATACAGATAGGGGGGCTATTTCCTAGGG
361 ---------+---------+---------+---------+---------+---------+ 420
    CCCCTGACTAAAAACCACAGAGAAGATTGTCGTATGTCTATCCCCCCGATAAAGGATCCC
```

FIG. 2B

```
         G  L  I  F  G| V  S  S  N  S  I  Q  I  G  G  L  F  P  R  G -

                     |_Mature N-Terminal

     GCGCCGATCAAGAATACAGTGCATTTCGAGTAGGGATGGTTCAGTTTTCCACTTCGGAGT
 421 ---------+---------+---------+---------+---------+---------+ 480
     CGCGGCTAGTTCTTATGTCACGTAAAGCTCATCCCTACCAAGTCAAAAGGTGAAGCCTCA

         A  D  Q  E  Y  S  A  F  R  V  G  M  V  Q  F  S  T  S  E  F -

     TCAGACTGACACCCCACATCGACAATTTGGAGGTGGCAAACAGCTTCGCAGTCACTAATG
 481 ---------+---------+---------+---------+---------+---------+ 540
     AGTCTGACTGTGGGGTGTAGCTGTTAAACCTCCACCGTTTGTCGAAGCGTCAGTGATTAC

         R  L  T  P  H  I  D  N  L  E  V  A  N  S  F  A  V  T  N  A -

     CTTTCTGCTCCCAGTTTTCGAGAGGAGTCTATGCTATTTTTGGATTTTATGACAAGAAGT
 541 ---------+---------+---------+---------+---------+---------+ 600
     GAAAGACGAGGGTCAAAAGCTCTCCTCAGATACGATAAAAACCTAAAATACTGTTCTTCA

         F  C  S  Q  F  S  R  G  V  Y  A  I  F  G  F  Y  D  K  K  S -

     CTGTAAATACCATCACATCATTTTGCGGAACACTCCACGTCTCCTTCATCACTCCCAGCT
 601 ---------+---------+---------+---------+---------+---------+ 660
     GACATTTATGGTAGTGTAGTAAAACGCCTTGTGAGGTGCAGAGGAAGTAGTGAGGGTCGA

         V  N  T  I  T  S  F  C  G  T  L  H  V  S  F  I  T  P  S  F -
```

FIG. 2C

```
    TCCCAACAGATGGCACACATCCATTTGTCATTCAGATGAGACCCGACCTCAAAGGAGCTC
661 ---------+---------+---------+---------+---------+---------+ 720
    AGGGTTGTCTACCGTGTGTAGGTAAACAGTAAGTCTACTCTGGGCTGGAGTTTCCTCGAG

     P  T  D  G  T  H  P  F  V  I  Q  M  R  P  D  L  K  G  A  L -

    TCCTTAGCTTGATTGAATACTATCAATGGGACAAGTTTGCATACCTCTATGACAGTGACA
721 ---------+---------+---------+---------+---------+---------+ 780
    AGGAATCGAACTAACTTATGATAGTTACCCTGTTCAAACGTATGGAGATACTGTCACTGT

     L  S  L  I  E  Y  Y  Q  W  D  K  F  A  Y  L  Y  D  S  D  R -

    GAGGCTTATCAACACTGCAAGCTGTGCTGGATTCTGCTGCTGAAAAGAAATGGCAAGTGA
781 ---------+---------+---------+---------+---------+---------+ 840
    CTCCGAATAGTTGTGACGTTCGACACGACCTAAGACGACGACTTTTCTTTACCGTTCACT

     G  L  S  T  L  Q  A  V  L  D  S  A  A  E  K  K  W  Q  V  T -

    CTGCTATCAATGTGGGAAACATTAACAATGACAAGAAAGATGAGATGTACCGATCACTTT
841 ---------+---------+---------+---------+---------+---------+ 900
    GACGATAGTTACACCCTTTGTAATTGTTACTGTTCTTTCTACTCTACATGGCTAGTGAAA

     A  I  N  V  G  N  I  N  N  D  K  K  D  E  M  Y  R  S  L  F -

    TTCAAGATCTGGAGTTAAAAAAGGAACGGCGTGTAATTCTGGACTGTGAAAGGGATAAAG
901 ---------+---------+---------+---------+---------+---------+ 960
    AAGTTCTAGACCTCAATTTTTTCCTTGCCGCACATTAAGACCTGACACTTTCCCTATTTC

     Q  D  L  E  L  K  K  E  R  R  V  I  L  D  C  E  R  D  K  V -
```

FIG. 2D

```
       TAAACGACATTGTAGACCAGGTTATTACCATTGGAAAACACGTTAAAGGTACCACTACA
 961   ---------+---------+---------+---------+---------+---------+ 1020
       ATTTGCTGTAACATCTGGTCCAATAATGGTAACCTTTTGTGCAATTTCCCATGGTGATGT

        N  D  I  V  D  Q  V  I  T  I  G  K  H  V  K  G  Y  H  Y  I -

       TCATTGCAAATCTGGGATTTACTGATGGAGACCTATTAAAAATCCAGTTTGGAGGTGCAA
 1021  ---------+---------+---------+---------+---------+---------+ 1080
       AGTAACGTTTAGACCCTAAATGACTACCTCTGGATAATTTTTAGGTCAAACCTCCACGTT

        I  A  N  L  G  F  T  D  G  D  L  L  K  I  Q  F  G  G  A  N -

       ATGTCTCTGGATTTCAGATAGTGGACTATGATGATTCGTTGGTATCTAAATTTATAGAAA
 1081  ---------+---------+---------+---------+---------+---------+ 1140
       TACAGAGACCTAAAGTCTATCACCTGATACTACTAAGCAACCATAGATTTAAATATCTTT

        V  S  G  F  Q  I  V  D  Y  D  D  S  L  V  S  K  F  I  E  R -

       GATGGTCAACACTGGAAGAAAAAGAATACCCTGGAGCTCACACAACAACAATTAAGTATA
 1141  ---------+---------+---------+---------+---------+---------+ 1200
       CTACCAGTTGTGACCTTCTTTTTCTTATGGGACCTCGAGTGTGTTGTTGTTAATTCATAT

        W  S  T  L  E  E  K  E  Y  P  G  A  H  T  T  T  I  K  Y  T -

       CTTCTGCTCTGACCTATGATGCCGTTCAAGTGATGACTGAAGCCTTCCGCAACCTAAGGA
 1201  ---------+---------+---------+---------+---------+---------+ 1260
       GAAGACGAGACTGGATACTACGGCAAGTTCACTACTGACTTCGGAAGGCGTTGGATTCCT

        S  A  L  T  Y  D  A  V  Q  V  M  T  E  A  F  R  N  L  R  K -
```

FIG. 2E

```
     AGCAAAGAATTGAAATCTCCCGAAGGGGAATGCAGGAGACTGTCTGGCAAACCCAGCAG
1261 ---------+---------+---------+---------+---------+---------+ 1320
     TCGTTTCTTAACTTTAGAGGGCTTCCCCTTACGTCCTCTGACAGACCGTTTGGGTCGTC

      Q  R  I  E  I  S  R  R  G  N  A  G  D  C  L  A  N  P  A  V -

     TGCCCTGGGGACAAGGTGTAGAAATAGAAAGGGCCCTCAAACAGGTTCAGGTTGAAGGTC
1321 ---------+---------+---------+---------+---------+---------+ 1380
     ACGGGACCCCTGTTCCACATCTTTATCTTTCCCGGGAGTTTGTCCAAGTCCAACTTCCAG

      P  W  G  Q  G  V  E  I  E  R  A  L  K  Q  V  Q  V  E  G  L -

     TCTCAGGAAATATAAAGTTTGACCAGAATGGAAAAAGAATAAACTATACAATTAACATCA
1381 ---------+---------+---------+---------+---------+---------+ 1440
     AGAGTCCTTTATATTTCAAACTGGTCTTACCTTTTTCTTATTTGATATGTTAATTGTAGT

      S  G  N  I  K  F  D  Q  N  G  K  R  I  N  Y  T  I  N  I  M -

     TGGAGCTCAAAACTAATGGGCCCCGGAAGATTGGCTACTGGAGTGAAGTGGACAAAATGG
1441 ---------+---------+---------+---------+---------+---------+ 1500
     ACCTCGAGTTTTGATTACCCGGGGCCTTCTAACCGATGACCTCACTTCACCTGTTTTACC

      E  L  K  T  N  G  P  R  K  I  G  Y  W  S  E  V  D  K  M  V -

     TTGTTACCCTTACTGAGCTCCCTTCTGGAAATGACACCTCTGGGCTTGAGAATAAGACTG
1501 ---------+---------+---------+---------+---------+---------+ 1560
     AACAATGGGAATGACTCGAGGGAAGACCTTTACTGTGGAGACCCGAACTCTTATTCTGAC

      V  T  L  T  E  L  P  S  G  N  D  T  S  G  L  E  N  K  T  V -
```

FIG. 2F

```
      TTGTTGTCACCACAATTTTGGAATCTCCGTATGTTATGATGAAGAAAAATCATGAAATGC
1561  ---------+---------+---------+---------+---------+---------+ 1620
      AACAACAGTGGTGTTAAAACCTTAGAGGCATACAATACTACTTCTTTTTAGTACTTTACG

       V  V  T  T  I  L  E  S  P  Y  V  M  M  K  K  N  H  E  M  L -

      TTGAAGGCAATGAGCGCTATGAGGGCTACTGTGTTGACCTGGCTGCAGAAATCGCCAAAC
1621  ---------+---------+---------+---------+---------+---------+ 1680
      AACTTCCGTTACTCGCGATACTCCCGATGACACAACTGGACCGACGTCTTTAGCGGTTTG

       E  G  N  E  R  Y  E  G  Y  C  V  D  L  A  A  E  I  A  K  H -

      ATTGTGGGTTCAAGTACAAGTTGACAATTGTTGGTGATGGCAAGTATGGGGCCAGGGATG
1681  ---------+---------+---------+---------+---------+---------+ 1740
      TAACACCCAAGTTCATGTTCAACTGTTAACAACCACTACCGTTCATACCCCGGTCCCTAC

       C  G  F  K  Y  K  L  T  I  V  G  D  G  K  Y  G  A  R  D  A -

      CAGACACGAAAATTTGGAATGGGATGGTTGGAGAACTTGTATATGGGAAAGCTGATATTG
1741  ---------+---------+---------+---------+---------+---------+ 1800
      GTCTGTGCTTTTAAACCTTACCCTACCAACCTCTTGAACATATACCCTTTCGACTATAAC

       D  T  K  I  W  N  G  M  V  G  E  L  V  Y  G  K  A  D  I  A -

      CAATTGCTCCATTAACTATTACCCTTGTGAGAGAAGAGGTGATTGACTTCTCAAAGCCCT
1801  ---------+---------+---------+---------+---------+---------+ 1860
      GTTAACGAGGTAATTGATAATGGGAACACTCTCTTCTCCACTAACTGAAGAGTTTCGGGA

       I  A  P  L  T  I  T  L  V  R  E  E  V  I  D  F  S  K  P  F -
```

FIG. 2G

```
     TCATGAGCCTCGGGATATCTATCATGATCAAGAAGCCTCAGAAGTCCAAACCAGGAGTGT
1861 ---------+---------+---------+---------+---------+---------+ 1920
     AGTACTCGGAGCCCTATAGATAGTACTAGTTCTTCGGAGTCTTCAGGTTTGGTCCTCACA

      M  S  L  G  I  S  I  M  I  K  K  P  Q  K  S  K  P  G  V  F -

     TTTCCTTTCTTGATCCTTTAGCCTATGAGATCTGGATGTGCATTGTTTTTGCCTACATTG
1921 ---------+---------+---------+---------+---------+---------+ 1980
     AAAGGAAAGAACTAGGAAATCGGATACTCTAGACCTACACGTAACAAAAACGGATGTAAC

      S  F  L  D  P  L  A  Y  E  I  W  M  C  I  V  F  A  Y  I  G -

     GGGTCAGTGTAGTTTTATTCCTGGTCAGCAGATTTAGCCCCTACGAGTGGCACACTGAGG
1981 ---------+---------+---------+---------+---------+---------+ 2040
     CCCAGTCACATCAAAATAAGGACCAGTCGTCTAAATCGGGGATGCTCACCGTGTGACTCC

      V  S  V  V  L  F  L  V  S  R  F  S  P  Y  E  W  H  T  E  E -

     AGTTTGAAGATGGAAGAGAAACACAAAGTAGTGAATCAACTAATGAATTTGGGATTTTTA
2041 ---------+---------+---------+---------+---------+---------+ 2100
     TCAAACTTCTACCTTCTCTTTGTGTTTCATCACTTAGTTGATTACTTAAACCCTAAAAAT

      F  E  D  G  R  E  T  Q  S  S  E  S  T  N  E  F  G  I  F  N -

     ATAGTCTCTGGTTTTCCTTGGGTGCCTTTATGCGGCAAGGATGCGATATTTCGCCAAGAT
2101 ---------+---------+---------+---------+---------+---------+ 2160
     TATCAGAGACCAAAAGGAACCCACGGAAATACGCCGTTCCTACGCTATAAAGCGGTTCTA

      S  L  W  F  S  L  G  A  F  M  R  Q  G  C  D  I  S  P  R  S -
```

FIG. 2H

```
      CCCTCTCTGGGCGCATTGTTGGAGGTGTGTGGTGGTTCTTTACCCTGATCATAATCTCCT
2161  ---------+---------+---------+---------+---------+---------+ 2220
      GGGAGAGACCCGCGTAACAACCTCCACACACCACCAAGAAATGGGACTAGTATTAGAGGA

       L  S  G  R  I  V  G  G  V  W  W  F  F  T  L  I  I  I  S  S -

      CCTACACGGCTAACTTAGCTGCCTTCCTGACTGTAGAGAGGATGGTGTCTCCCATCGAAA
2221  ---------+---------+---------+---------+---------+---------+ 2280
      GGATGTGCCGATTGAATCGACGGAAGGACTGACATCTCTCCTACCACAGAGGGTAGCTTT

       Y  T  A  N  L  A  A  F  L  T  V  E  R  M  V  S  P  I  E  S -

      GTGCTGAGGATCTTTCTAAGCAAACAGAAATTGCTTATGGAACATTAGACTCTGGCTCCA
2281  ---------+---------+---------+---------+---------+---------+ 2340
      CACGACTCCTAGAAAGATTCGTTTGTCTTTAACGAATACCTTGTAATCTGAGACCGAGGT

       A  E  D  L  S  K  Q  T  E  I  A  Y  G  T  L  D  S  G  S  T -

      CTAAAGAGTTTTTCAGGAGATCTAAAATTGCAGTGTTTGATAAAATGTGGACCTACATGC
2341  ---------+---------+---------+---------+---------+---------+ 2400
      GATTTCTCAAAAAGTCCTCTAGATTTTAACGTCACAAACTATTTTACACCTGGATGTACG

       K  E  F  F  R  R  S  K  I  A  V  F  D  K  M  W  T  Y  M  R -

      GGAGTGCGGAGCCCTCTGTGTTTGTGAGGACTACGGCCGAAGGGGTGGCTAGAGTGCGGA
2401  ---------+---------+---------+---------+---------+---------+ 2460
      CCTCACGCCTCGGGAGACACAAACACTCCTGATGCCGGCTTCCCCACCGATCTCACGCCT

       S  A  E  P  S  V  F  V  R  T  T  A  E  G  V  A  R  V  R  K -
```

FIG. 2I

```
     AGTCCAAAGGGAAATATGCCTACTTGTTGGAGTCCACGATGAACGAGTACATTGAGCAAA
2461 ---------+---------+---------+---------+---------+---------+ 2520
     TCAGGTTTCCCTTTATACGGATGAACAACCTCAGGTGCTACTTGCTCATGTAACTCGTTT

      S  K  G  K  Y  A  Y  L  L  E  S  T  M  N  E  Y  I  E  Q  R -

     GGAAGCCTTGCGACACCATGAAAGTTGGTGGAAACCTGGATTCCAAAGGCTATGGCATCG
2521 ---------+---------+---------+---------+---------+---------+ 2580
     CCTTCGGAACGCTGTGGTACTTTCAACCACCTTTGGACCTAAGGTTTCCGATACCGTAGC

      K  P  C  D  T  M  K  V  G  G  N  L  D  S  K  G  Y  G  I  A -

     CAACACCTAAAGGATCCTCATTAGGAACCCCAGTAAATCTTGCAGTATTGAAACTCAGTG
2581 ---------+---------+---------+---------+---------+---------+ 2640
     GTTGTGGATTTCCTAGGAGTAATCCTTGGGGTCATTTAGAACGTCATAACTTTGAGTCAC

      T  P  K  G  S  S  L  G  T  P  V  N  L  A  V  L  K  L  S  E -

     AGCAAGGCGTCTTAGACAAGCTGAAAAACAAATGGTGGTACGATAAAGGTGAATGTGGAG
2641 ---------+---------+---------+---------+---------+---------+ 2700
     TCGTTCCGCAGAATCTGTTCGACTTTTTGTTTACCACCATGCTATTTCCACTTACACCTC

      Q  G  V  L  D  K  L  K  N  K  W  W  Y  D  K  G  E  C  G  A -

     CCAAGGACTCTGGAAGTAAGGAAAAGACCAGTGCCCTCAGTCTGAGCAACGTTGCTGGAG
2701 ---------+---------+---------+---------+---------+---------+ 2760
     GGTTCCTGAGACCTTCATTCCTTTTCTGGTCACGGGAGTCAGACTCGTTGCAACGACCTC

      K  D  S  G  S  K  E  K  T  S  A  L  S  L  S  N  V  A  G  V -
```

FIG. 2J

```
      TATTCTACATCCTTGTCGGGGGCCTTGGTTTGGCAATGCTGGTGGCTTTGATTGAGTTCT
2761 ---------+---------+---------+---------+---------+---------+ 2820
      ATAAGATGTAGGAACAGCCCCCGGAACCAAACCGTTACGACCACCGAAACTAACTCAAGA

        F  Y  I  L  V  G  G  L  G  L  A  M  L  V  A  L  I  E  F  C -

      GTTACAAGTCAAGGGCCGAGGCGAAACGAATGAAGGTGGCAAAGAATGCACAGAATATTA
2821 ---------+---------+---------+---------+---------+---------+ 2880
      CAATGTTCAGTTCCCGGCTCCGCTTTGCTTACTTCCACCGTTTCTTACGTGTCTTATAAT

        Y  K  S  R  A  E  A  K  R  M  K  V  A  K  N  A  Q  N  I  N -

                      EcoRI
                      |
      ACCCATCTTCCTCGCAGAATTCACAGAATTTTGCAACTTATAAGGAAGGTTACAACGTAT
2881 ---------+---------+---------+---------+---------+---------+ 2940
      TGGGTAGAAGGAGCGTCTTAAGTGTCTTAAAACGTTGAATATTCCTTCCAATGTTGCATA

        P  S  S  S  Q  N  S  Q  N  F  A  T  Y  K  E  G  Y  N  V  Y -

      ATGGCATCGAAAGTGTTAAAATTTAGGGGATGACCTTGAATGATGCCATGAGGAACAAGG
2941 ---------+---------+---------+---------+---------+---------+ 3000
      TACCGTAGCTTTCACAATTTTAAATCCCCTACTGGAACTTACTACGGTACTCCTTGTTCC

        G  I  E  S  V  K  I  *

      CAAGGCTGTCAATTACAGGAAGTACTGGAGAAAATGGACGTGTTATGACTCCAGAATTTC
3001 ---------+---------+---------+---------+---------+---------+ 3060
      GTTCCGACAGTTAATGTCCTTCATGACCTCTTTTACCTGCACAATACTGAGGTCTTAAAG
```

FIG. 2K

```
                                                                     EcoRI
      CCAAAGCNGTGCATGCTGTCCCTTACGTGAGTCCTGGCATGGGAATGAATGTCAGTGTGA
3061  ---------+---------+---------+---------+---------+---------+ 3120
      GGTTTCGNCACGTACGACAGGGAATGCACTCAGGACCGTACCCTTACTTACAGTCACACT

      CTGATCTCTCGTGATTGATAAGAACCTTTTGAGTGCCTTACACAATGGTTTTCTTGTGTG
3121  ---------+---------+---------+---------+---------+---------+ 3180
      GACTAGAGAGCACTAACTATTCTTGGAAAACTCACGGAATGTGTTACCAAAAGAACACAC

                                                                EcoRI
                                                                  |
      TTTATTGTCAAAGTGGTGAGAGGCATCCAGTATCTTGAAGACTTTTCTTTCAGCCAAGAA
3181  ---------+---------+---------+---------+---------+---------+ 3240
      AAATAACAGTTTCACCACTCTCCGTAGGTCATAGAACTTCTGAAAAGAAAGTCGGTTCTT

      TTCTTAAATATGTGGAGTTCATCTTGAATTGTAAGGAATGATTAATTAAAACACAACATC
3241  ---------+---------+---------+---------+---------+---------+ 3300
      AAGAATTTATACACCTCAAGTAGAACTTAACATTCCTTACTAATTAATTTTGTGTTGTAG

      TTTTTCTACTCGAGTTACAGACAAAGCGTGGTGGACATGCACAGCTAACATGGAAGTACT
3301  ---------+---------+---------+---------+---------+---------+ 3360
      AAAAAGATGAGCTCAATGTCTGTTTCGCACCACCTGTACGTGTCGATTGTACCTTCATGA

      ATAATTTACCTGAAGTCTTTGTACAGACAACAAACCTGTTTCTGCAG
3361  ---------+---------+---------+---------+------- 3407
      TATTAAATGGACTTCAGAAACATGTCTGTTGTTTGGACAAAGACGTC
```

FIG. 3A

```
      EcoRI
      |
      gaattcctgacgactcctgagttgcgcccatgctcttgtcagcttcgttttaggcgtagc
    1 ---------+---------+---------+---------+---------+---------+ 60
      cttaaggactgctgaggactcaacgcgggtacgagaacagtcgaagcaaaatccgcatcg

      atggccaggcagaagaaaatggggcaaagcgtgctccgggcggtcttcttttagtcctg
   61 ---------+---------+---------+---------+---------+---------+ 120
      taccggtccgtcttcttttaccccgtttcgcacgaggcccgccagaagaaaatcaggac

                        M  G  Q  S  V  L  R  A  V  F  F  L  V  L  -

      gggcttttgggtcattctcacggaggattccccaacaccatcagcataggtggacttttc
  121 ---------+---------+---------+---------+---------+---------+ 180
      cccgaaaacccagtaagagtgcctcctaaggggttgtggtagtcgtatccacctgaaaag

      G  L  L  G  H  S  H  G  G  F  P  N  T  I  S  I  G  G  L  F  -

                      |_Mature N-Terminal

      atgagaaacacagtgcaggagcacagcgctttccgctttgccgtgcagttatacaacacc
  181 ---------+---------+---------+---------+---------+---------+ 240
      tactctttgtgtcacgtcctcgtgtcgcgaaaggcgaaacggcacgtcaatatgttgtgg

      M  R  N  T  V  Q  E  H  S  A  F  R  F  A  V  Q  L  Y  N  T  -

      aaccagaacaccaccgagaagcccttccatttgaattaccacgtagatcacttggattcc
  241 ---------+---------+---------+---------+---------+---------+ 300
      ttggtcttgtggtggctcttcgggaaggtaaacttaatggtgcatctagtgaacctaagg

      N  Q  N  T  T  E  K  P  F  H  L  N  Y  H  V  D  H  L  D  S  -
```

FIG. 3B

```
     tccaatagtttttccgtgacaaatgctttctgctcccagttctcgagaggggtgtatgcc
301  ---------+---------+---------+---------+---------+---------+ 360
     aggttatcaaaaaggcactgtttacgaaagacgagggtcaagagctctccccacatacgg

     S  N  S  F  S  V  T  N  A  F  C  S  Q  F  S  R  G  V  Y  A   -

     atctttggattctatgaccagatgtcaatgaacaccctgacctccttctgtggggccctg
361  ---------+---------+---------+---------+---------+---------+ 420
     tagaaacctaagatactggtctacagttacttgtgggactggaggaagacaccccgggac

     I  F  G  F  Y  D  Q  M  S  M  N  T  L  T  S  F  C  G  A  L   -

     cacacatcctttgttacgcctagcttccccactgacgcagatgtgcagtttgtcatccag
421  ---------+---------+---------+---------+---------+---------+ 480
     gtgtgtaggaaacaatgcggatcgaaggggtgactgcgtctacacgtcaaacagtaggtc

     H  T  S  F  V  T  P  S  F  P  T  D  A  D  V  Q  F  V  I  Q   -

     atgcgcccagccttgaagggcgctattctgagtcttctgggtcattacaagtgggagaag
481  ---------+---------+---------+---------+---------+---------+ 540
     tacgcgggtcggaacttcccgcgataagactcagaagacccagtaatgttcaccctcttc

     M  R  P  A  L  K  G  A  I  L  S  L  L  G  H  Y  K  W  E  K   -

     tttgtgtacctctatgacacagaacgaggattttccatcctccaagcgattatggaagca
541  ---------+---------+---------+---------+---------+---------+ 600
     aaacacatggagatactgtgtcttgctcctaaaaggtaggaggttcgctaataccttcgt

     F  V  Y  L  Y  D  T  E  R  G  F  S  I  L  Q  A  I  M  E  A   -
```

FIG. 3C

```
    gcagtgcaaaacaactggcaagtaacagcaaggtctgtgggaaacataaaggacgtccaa
601 ---------+---------+---------+---------+---------+---------+ 660
    cgtcacgttttgttgaccgttcattgtcgttccagacaccctttgtatttcctgcaggtt

    A  V  Q  N  N  W  Q  V  T  A  R  S  V  G  N  I  K  D  V  Q   -

    EcoRI
    |
    gaattcaggcgcatcattgaagaaatggacaggaggcaggaaaagcgatacttgattgac
661 ---------+---------+---------+---------+---------+---------+ 720
    cttaagtccgcgtagtaacttctttacctgtcctccgtccttttcgctatgaactaactg

    E  F  R  R  I  I  E  E  M  D  R  R  Q  E  K  R  Y  L  I  D   -

    tgcgaagtcgaaaggattaacacaattttggaacaggttgtgatcctagggaaacactca
721 ---------+---------+---------+---------+---------+---------+ 780
    acgcttcagctttcctaattgtgttaaaaccttgtccaacactaggatccctttgtgagt

    C  E  V  E  R  I  N  T  I  L  E  Q  V  V  I  L  G  K  H  S   -

    agaggttatcactacatgctcgctaacctgggttttactgatattttactggaaagagtc
781 ---------+---------+---------+---------+---------+---------+ 840
    tctccaatagtgatgtacgagcgattggacccaaaatgactataaaatgacctttctcag

    R  G  Y  H  Y  M  L  A  N  L  G  F  T  D  I  L  L  E  R  V   -

    atgcatgggggagccaacattacaggtttccagattgtcaacaatgaaaaccctatggtt
841 ---------+---------+---------+---------+---------+---------+ 900
    tacgtaccccctcggttgtaatgtccaaaggtctaacagttgttacttttgggataccaa

    M  H  G  G  A  N  I  T  G  F  Q  I  V  N  N  E  N  P  M  V   -
```

FIG. 3D

```
                                                      EcoRI
                                                        |
     cagcagttcatacagcgctgggtgaggctggatgaaagggaattccctgaagccaagaat
 901 ---------+---------+---------+---------+---------+---------+ 960
     gtcgtcaagtatgtcgcgacccactccgacctactttcccttaaggacttcggttctta

     Q  Q  F  I  Q  R  W  V  R  L  D  E  R  E  F  P  E  A  K  N  -

                                                          HindIII
                                                             |
     gcaccactaaagtatacatctgcattgacacacgacgcaatactggtcatagcagaagct
 961 ---------+---------+---------+---------+---------+---------+ 1020
     cgtggtgatttcatatgtagacgtaactgtgtgctgcgttatgaccagtatcgtcttcga

     A  P  L  K  Y  T  S  A  L  T  H  D  A  I  L  V  I  A  E  A  -

     ttccgctacctgaggaggcagcgagtagatgtgtcccggagaggaagtgctggagactgc
1021 ---------+---------+---------+---------+---------+---------+ 1080
     aaggcgatggactcctccgtcgctcatctacacagggcctctccttcacgacctctgacg

     F  R  Y  L  R  R  Q  R  V  D  V  S  R  R  G  S  A  G  D  C  -

     ttagcaaatcctgctgtgccctggagtcaaggaattgatattgagagagctctgaaaatg
1081 ---------+---------+---------+---------+---------+---------+ 1140
     aatcgtttaggacgacacgggacctcagttccttaactataactctctcgagacttttac

     L  A  N  P  A  V  P  W  S  Q  G  I  D  I  E  R  A  L  K  M  -
```

FIG. 3E

```
       gtgcaagtacaaggaatgactggaaatattcaatttgacacttatggacgtaggacaaat
1141 ---------+---------+---------+---------+---------+---------+ 1200
       cacgttcatgttccttactgacctttataagttaaactgtgaatacctgcatcctgttta

       V  Q  V  Q  G  M  T  G  N  I  Q  F  D  T  Y  G  R  R  T  N   -

       tataccatcgatgtgtatgaaatgaaagtcagtggctctcgaaaagctggctactggaac
1201 ---------+---------+---------+---------+---------+---------+ 1260
       atatggtagctacacatactttactttcagtcaccgagagcttttcgaccgatgaccttg

       Y  T  I  D  V  Y  E  M  K  V  S  G  S  R  K  A  G  Y  W  N   -

       gagtatgaaaggtttgtgcctttctcagatcagcaaatcagcaatgacagtgcatcctca
1261 ---------+---------+---------+---------+---------+---------+ 1320
       ctcatactttccaaacacggaaagagtctagtcgtttagtcgttactgtcacgtaggagt

       E  Y  E  R  F  V  P  F  S  D  Q  Q  I  S  N  D  S  A  S  S   -

       gagaatcggaccatagtagtgactaccattctggaatcaccatatgtaatgtacaagaag
1321 ---------+---------+---------+---------+---------+---------+ 1380
       ctcttagcctggtatcatcactgatggtaagaccttagtggtatacattacatgttcttc

       E  N  R  T  I  V  V  T  T  I  L  E  S  P  Y  V  M  Y  K  K   -

       aaccatgagcaactggaaggaaatgaacgatatgaaggctattgtgtagacctagcctat
1381 ---------+---------+---------+---------+---------+---------+ 1440
       ttggtactcgttgaccttcctttacttgctatacttccgataacacatctggatcggata

       N  H  E  Q  L  E  G  N  E  R  Y  E  G  Y  C  V  D  L  A  Y   -
```

FIG. 3F

```
     gaaatagccaaacatgtaaggatcaaatacaaattgtccatcgttggtgacgggaaatat
1441 ---------+---------+---------+---------+---------+---------+ 1500
     ctttatcggtttgtacattcctagtttatgtttaacaggtagcaaccactgccctttata

     E  I  A  K  H  V  R  I  K  Y  K  L  S  I  V  G  D  G  K  Y   -

     ggtgcaagggatccagagactaaaatatggaacggcatggttggggaacttgtctatggg
1501 ---------+---------+---------+---------+---------+---------+ 1560
     ccacgttccctaggtctctgattttataccttgccgtaccaaccccttgaacagataccc

     G  A  R  D  P  E  T  K  I  W  N  G  M  V  G  E  L  V  Y  G   -

     agagctgatatagctgttgctccactcactataacattggtccgtgaagaagtcatagat
1561 ---------+---------+---------+---------+---------+---------+ 1620
     tctcgactatatcgacaacgaggtgagtgatattgtaaccaggcacttcttcagtatcta

     R  A  D  I  A  V  A  P  L  T  I  T  L  V  R  E  E  V  I  D   -

     ttttcaaagccattaatgagcctgggcatctccatcatgataaagaagcctcagaaatca
1621 ---------+---------+---------+---------+---------+---------+ 1680
     aaaagtttcggtaattactcggacccgtagaggtagtactatttcttcggagtctttagt

     F  S  K  P  L  M  S  L  G  I  S  I  M  I  K  K  P  Q  K  S   -

     aaaccaggcgtattctcatttctggatcccctggcttatgaaatctggatgtgcattgtc
1681 ---------+---------+---------+---------+---------+---------+ 1740
     tttggtccgcataagagtaaagacctaggggaccgaatactttagacctacacgtaacag

     K  P  G  V  F  S  F  L  D  P  L  A  Y  E  I  W  M  C  I  V   -
```

FIG. 3G

```
      tttgcttacattggagtcagcgtagttcttttcctagtcagcaggttcagtccttatgaa
1741  ---------+---------+---------+---------+---------+---------+ 1800
      aaacgaatgtaacctcagtcgcatcaagaaaaggatcagtcgtccaagtcaggaatactt

      F  A  Y  I  G  V  S  V  V  L  F  L  V  S  R  F  S  P  Y  E  -

      tggcacttggaagacaacaatgaagaacctcgtgacccacaaagtcctcctgatcctcca
1801  ---------+---------+---------+---------+---------+---------+ 1860
      accgtgaaccttctgttgttacttcttggagcactgggtgtttcaggaggactaggaggt

      W  H  L  E  D  N  N  E  E  P  R  D  P  Q  S  P  P  D  P  P  -

      aatgaatttggaatatttaacagtctttggttttccttgggtgcctttatgcagcaagga
1861  ---------+---------+---------+---------+---------+---------+ 1920
      ttacttaaaccttataaattgtcagaaaccaaaaggaacccacggaaatacgtcgttcct

      N  E  F  G  I  F  N  S  L  W  F  S  L  G  A  F  M  Q  Q  G  -

      tgtgatatttctccaagatcactctccgggcgcattgttggaggggtttggtggttcttc
1921  ---------+---------+---------+---------+---------+---------+ 1980
      acactataaagaggttctagtgagaggcccgcgtaacaacctccccaaaccaccaagaag

      C  D  I  S  P  R  S  L  S  G  R  I  V  G  G  V  W  W  F  F  -

      accctgatcataatttcttcctatactgccaatctcgctgctttcctgactgtggagagg
1981  ---------+---------+---------+---------+---------+---------+ 2040
      tgggactagtattaaagaaggatatgacggttagagcgacgaaaggactgacacctctcc

      T  L  I  I  I  S  S  Y  T  A  N  L  A  A  F  L  T  V  E  R  -
```

FIG. 3H

```
     atggtttctcccatagagagtgctgaagacttagctaaacagactgaaattgcatatggg
2041 ---------+---------+---------+---------+---------+---------+ 2100
     taccaaagagggtatctctcacgacttctgaatcgatttgtctgactttaacgtataccc

     M  V  S  P  I  E  S  A  E  D  L  A  K  Q  T  E  I  A  Y  G  -

     accctggactccggttcaacaaaagaatttttcagaagatccaaaattgctgtgtacgag
2101 ---------+---------+---------+---------+---------+---------+ 2160
     tgggacctgaggccaagttgttttcttaaaaagtcttctaggttttaacgacacatgctc

     T  L  D  S  G  S  T  K  E  F  F  R  R  S  K  I  A  V  Y  E  -

     aaaatgtggtcttacatgaaatcagcggagccatctgtgtttaccaaaacaacagcagac
2161 ---------+---------+---------+---------+---------+---------+ 2220
     ttttacaccagaatgtactttagtcgcctcggtagacacaaatggttttgttgtcgtctg

     K  M  W  S  Y  M  K  S  A  E  P  S  V  F  T  K  T  T  A  D  -

     ggagtggcccgagtgcgaaagtccaagggaaagttcgccttcctgctggagtcaaccatg
2221 ---------+---------+---------+---------+---------+---------+ 2280
     cctcaccgggctcacgctttcaggttccctttcaagcggaaggacgacctcagttggtac

     G  V  A  R  V  R  K  S  K  G  K  F  A  F  L  L  E  S  T  M  -

     aatgagtacattgagcagagaaaaccatgtgatacgatgaaagttggtggaaatctggat
2281 ---------+---------+---------+---------+---------+---------+ 2340
     ttactcatgtaactcgtctcttttggtacactatgctactttcaaccacctttagaccta

     N  E  Y  I  E  Q  R  K  P  C  D  T  M  K  V  G  G  N  L  D  -
```

FIG. 3I

```
     tccaaaggctatggtgtggcaacccctaaaggctcagcattaggaaatgctgttaacctg
2341 ---------+---------+---------+---------+---------+---------+ 2400
     aggtttccgataccacaccgttggggatttccgagtcgtaatcctttacgacaattggac

     S  K  G  Y  G  V  A  T  P  K  G  S  A  L  G  N  A  V  N  L
                                StuI
                                 |
     gcagtattaaaactgaatgagcaaggcctcttggacaaattgaaaaacaaatggtggtac
2401 ---------+---------+---------+---------+---------+---------+ 2460
     cgtcataattttgacttactcgttccggagaacctgtttaactttttgtttaccaccatg

     A  V  L  K  L  N  E  Q  G  L  L  D  K  L  K  N  K  W  W  Y   -

     gacaaaggagagtgcggcagcggggcggtgactccaaggacaagaccagcgctctgagc
2461 ---------+---------+---------+---------+---------+---------+ 2520
     ctgtttcctctcacgccgtcgccccgccactgaggttcctgttctggtcgcgagactcg

     D  K  G  E  C  G  S  G  G  G  D  S  K  D  K  T  S  A  L  S   -

     ctgagcaatgtggcaggcgttttctatatacttgtcggaggtctggggctggccatgatg
2521 ---------+---------+---------+---------+---------+---------+ 2580
     gactcgttacaccgtccgcaaaagatatatgaacagcctccagaccccgaccggtactac

     L  S  N  V  A  G  V  F  Y  I  L  V  G  G  L  G  L  A  M  M   -
```

FIG. 3J

```
                         EcoRI
                           |
      gtggctttgatagaattctgttacaaatcacgggcagagtccaaacgcatgaaactcaca
 2581 ---------+---------+---------+---------+---------+---------+ 2640
      caccgaaactatcttaagacaatgtttagtgcccgtctcaggtttgcgtactttgagtgt

      V  A  L  I  E  F  C  Y  K  S  R  A  E  S  K  R  M  K  L  T   -

      aagaacacccaaaactttaagcctgctcctgccaccaacactcagaattatgctacatac
 2641 ---------+---------+---------+---------+---------+---------+ 2700
      ttcttgtgggttttgaaattcggacgaggacggtggttgtgagtcttaatacgatgtatg

      K  N  T  Q  N  F  K  P  A  P  A  T  N  T  Q  N  Y  A  T  Y   -

      agagaaggctacaacgtgtatggaacagagagtgttaagatctagggatcccttggaatt
 2701 ---------+---------+---------+---------+---------+---------+ 2760
      tctcttccgatgttgcacataccttgtctctcacaattctagatccctagggaacctta a

      R  E  G  Y  N  V  Y  G  T  E  S  V  K  I  *                  -

      c
 2761 - 2761
      g
```

FIG. 4A

```
          EcoRI
          |
           GAATTCCTGACGACTCCTGAGTTGCGCCCATGCTCTTGTCAGCTTCGTTTTAGGCGTAGC
        1 ---------+---------+---------+---------+---------+---------+ 60
          CTTAAGGACTGCTGAGGACTCAACGCGGGTACGAGAACAGTCGAAGCAAAATCCGCATCG

          ATGGCCAGGCAGAAGAAAATGGGGCAAAGCGTGCTCCGGGCGGTCTTCTTTTTAGTCCTG
       61 ---------+---------+---------+---------+---------+---------+ 120
          TACCGGTCCGTCTTCTTTTACCCCGTTTCGCACGAGGCCCGCCAGAAGAAAAATCAGGAC

                                M  G  Q  S  V  L  R  A  V  F  F  L  V  L  -

          GGGCTTTTGGGTCATTCTCACGGAGGATTCCCCAACACCATCAGCATAGGTGGACTTTTC
      121 ---------+---------+---------+---------+---------+---------+ 180
          CCCGAAAACCCAGTAAGAGTGCCTCCTAAGGGGTTGTGGTAGTCGTATCCACCTGAAAAG

a         G  L  L  G  H  S  H  G  G  F  P  N  T  I  S  I  G  G  L  F  -

                        |_Mature N-Terminal

          ATGAGAAACACAGTGCAGGAGCACAGCGCTTTCCGCTTTGCCGTGCAGTTATACAACACC
      181 ---------+---------+---------+---------+---------+---------+ 240
          TACTCTTTGTGTCACGTCCTCGTGTCGCGAAAGGCGAAACGGCACGTCAATATGTTGTGG

a         M  R  N  T  V  Q  E  H  S  A  F  R  F  A  V  Q  L  Y  N  T  -

          AACCAGAACACCACCGAGAAGCCCTTCCATTTGAATTACCACGTAGATCACTTGGATTCC
      241 ---------+---------+---------+---------+---------+---------+ 300
          TTGGTCTTGTGGTGGCTCTTCGGGAAGGTAAACTTAATGGTGCATCTAGTGAACCTAAGG

a         N  Q  N  T  T  E  K  P  F  H  L  N  Y  H  V  D  H  L  D  S  -
```

FIG. 4B

```
    TCCAATAGTTTTTCCGTGACAAATGCTTTCTGCTCCCAGTTCTCGAGAGGGGTGTATGCC
301 ---------+---------+---------+---------+---------+---------+ 360
    AGGTTATCAAAAAGGCACTGTTTACGAAAGACGAGGGTCAAGAGCTCTCCCCACATACGG

a   S  N  S  F  S  V  T  N  A  F  C  S  Q  F  S  R  G  V  Y  A  -

    ATCTTTGGATTCTATGACCAGATGTCAATGAACACCCTGACCTCCTTCTGTGGGGCCCTG
361 ---------+---------+---------+---------+---------+---------+ 420
    TAGAAACCTAAGATACTGGTCTACAGTTACTTGTGGGACTGGAGGAAGACACCCCGGGAC

a   I  F  G  F  Y  D  Q  M  S  M  N  T  L  T  S  F  C  G  A  L  -

    CACACATCCTTTGTTACGCCTAGCTTCCCCACTGACGCAGATGTGCAGTTTGTCATCCAG
421 ---------+---------+---------+---------+---------+---------+ 480
    GTGTGTAGGAAACAATGCGGATCGAAGGGGTGACTGCGTCTACACGTCAAACAGTAGGTC

a   H  T  S  F  V  T  P  S  F  P  T  D  A  D  V  Q  F  V  I  Q  -
```

FIG. 4C

```
        ATGCGCCCAGCCTTGAAGGGCGCTATTCTGAGTCTTCTGGGTCATTACAAGTGGGAGAAG
   481  ---------+---------+---------+---------+---------+---------+ 540
        TACGCGGGTCGGAACTTCCCGCGATAAGACTCAGAAGACCCAGTAATGTTCACCCTCTTC

a       M  R  P  A  L  K  G  A  I  L  S  L  L  G  H  Y  K  W  E  K  -

        TTTGTGTACCTCTATGACACAGAACGAGGATTTTCCATCCTCCAAGCGATTATGGAAGCA
   541  ---------+---------+---------+---------+---------+---------+ 600
        AAACACATGGAGATACTGTGTCTTGCTCCTAAAAGGTAGGAGGTTCGCTAATACCTTCGT

a       F  V  Y  L  Y  D  T  E  R  G  F  S  I  L  Q  A  I  M  E  A  -

        GCAGTGCAAAACAACTGGCAAGTAACAGCAAGGTCTGTGGGAAACATAAAGGACGTCCAA
   601  ---------+---------+---------+---------+---------+---------+ 660
        CGTCACGTTTTGTTGACCGTTCATTGTCGTTCCAGACACCCTTTGTATTTCCTGCAGGTT

a       A  V  Q  N  N  W  Q  V  T  A  R  S  V  G  N  I  K  D  V  Q  -

        EcoRI
        |
        GAATTCAGGCGCATCATTGAAGAAATGGACAGGAGGCAGGAAAAGCGATACTTGATTGAC
   661  ---------+---------+---------+---------+---------+---------+ 720
        CTTAAGTCCGCGTAGTAACTTCTTTACCTGTCCTCCGTCCTTTTCGCTATGAACTAACTG

a       E  F  R  R  I  I  E  E  M  D  R  R  Q  E  K  R  Y  L  I  D  -

        TGCGAAGTCGAAAGGATTAACACAATTTTGGAACAGGTTGTGATCCTAGGGAAACACTCA
   721  ---------+---------+---------+---------+---------+---------+ 780
        ACGCTTCAGCTTTCCTAATTGTGTTAAAACCTTGTCCAACACTAGGATCCCTTTGTGAGT
```

FIG. 4D

```
a        C  E  V  E  R  I  N  T  I  L  E  Q  V  V  I  L  G  K  H  S  -

         AGAGGTTATCACTACATGCTCGCTAACCTGGGTTTTACTGATATTTTACTGGAAAGAGTC
     781 ---------+---------+---------+---------+---------+---------+ 840
         TCTCCAATAGTGATGTACGAGCGATTGGACCCAAAATGACTATAAAATGACCTTTCTCAG

a        R  G  Y  H  Y  M  L  A  N  L  G  F  T  D  I  L  L  E  R  V  -

         ATGCATGGGGGAGCCAACATTACAGGTTTCCAGATTGTCAACAATGAAAACCCTATGGTT
     841 ---------+---------+---------+---------+---------+---------+ 900
         TACGTACCCCCTCGGTTGTAATGTCCAAAGGTCTAACAGTTGTTACTTTTGGGATACCAA

a        M  H  G  G  A  N  I  T  G  F  Q  I  V  N  N  E  N  P  M  V  -

                                                 EcoRI
                                                 |
         CAGCAGTTCATACAGCGCTGGGTGAGGCTGGATGAAAGGGAATTCCCTGAAGCCAAGAAT
     901 ---------+---------+---------+---------+---------+---------+ 960
         GTCGTCAAGTATGTCGCGACCCACTCCGACCTACTTTCCCTTAAGGGACTTCGGTTCTTA

a        Q  Q  F  I  Q  R  W  V  R  L  D  E  R  E  F  P  E  A  K  N  -

                                                              HindIII
                                                              |
         GCACCACTAAAGTATACATCTGCATTGACACACGACGCAATACTGGTCATAGCAGAAGCT
     961 ---------+---------+---------+---------+---------+---------+ 1020
         CGTGGTGATTTCATATGTAGACGTAACTGTGTGCTGCGTTATGACCAGTATCGTCTTCGA

a        A  P  L  K  Y  T  S  A  L  T  H  D  A  I  L  V  I  A  E  A  -
```

FIG. 4E

```
          TTCCGCTACCTGAGGAGGCAGCGAGTAGATGTGTCCCGGAGAGGAAGTGCTGGAGACTGC
     1021 ---------+---------+---------+---------+---------+---------+ 1080
          AAGGCGATGGACTCCTCCGTCGCTCATCTACACAGGGCCTCTCCTTCACGACCTCTGACG

a         F  R  Y  L  R  R  Q  R  V  D  V  S  R  R  G  S  A  G  D  C  -

          TTAGCAAATCCTGCTGTGCCCTGGAGTCAAGGAATTGATATTGAGAGAGCTCTGAAAATG
     1081 ---------+---------+---------+---------+---------+---------+ 1140
          AATCGTTTAGGACGACACGGGACCTCAGTTCCTTAACTATAACTCTCTCGAGACTTTTAC

a         L  A  N  P  A  V  P  W  S  Q  G  I  D  I  E  R  A  L  K  M  -

          GTGCAAGTACAAGGAATGACTGGAAATATTCAATTTGACACTTATGGACGTAGGACAAAT
     1141 ---------+---------+---------+---------+---------+---------+ 1200
          CACGTTCATGTTCCTTACTGACCTTTATAAGTTAAACTGTGAATACCTGCATCCTGTTTA

a         V  Q  V  Q  G  M  T  G  N  I  Q  F  D  T  Y  G  R  R  T  N  -

          TATACCATCGATGTGTATGAAATGAAAGTCAGTGGCTCTCGAAAAGCTGGCTACTGGAAC
     1201 ---------+---------+---------+---------+---------+---------+ 1260
          ATATGGTAGCTACACATACTTTACTTTCAGTCACCGAGAGCTTTTCGACCGATGACCTTG

a         Y  T  I  D  V  Y  E  M  K  V  S  G  S  R  K  A  G  Y  W  N  -

          GAGTATGAAAGGTTTGTGCCTTTCTCAGATCAGCAAATCAGCAATGACAGTGCATCCTCA
     1261 ---------+---------+---------+---------+---------+---------+ 1320
          CTCATACTTTCCAAACACGGAAAGAGTCTAGTCGTTTAGTCGTTACTGTCACGTAGGAGT

a         E  Y  E  R  F  V  P  F  S  D  Q  Q  I  S  N  D  S  A  S  S  -
```

FIG. 4F

```
      GAGAATCGGACCATAGTAGTGACTACCATTCTGGAATCACCATATGTAATGTACAAGAAG
 1321 ---------+---------+---------+---------+---------+---------+ 1380
      CTCTTAGCCTGGTATCATCACTGATGGTAAGACCTTAGTGGTATACATTACATGTTCTTC

a     E  N  R  T  I  V  V  T  T  I  L  E  S  P  Y  V  M  Y  K  K  -

      AACCATGAGCAACTGGAAGGAAATGAACGATATGAAGGCTATTGTGTAGACCTAGCCTAT
 1381 ---------+---------+---------+---------+---------+---------+ 1440
      TTGGTACTCGTTGACCTTCCTTTACTTGCTATACTTCCGATAACACATCTGGATCGGATA

a     N  H  E  Q  L  E  G  N  E  R  Y  E  G  Y  C  V  D  L  A  Y  -

      GAAATAGCCAAACATGTAAGGATCAAATACAAATTGTCCATCGTTGGTGACGGGAAATAT
 1441 ---------+---------+---------+---------+---------+---------+ 1500
      CTTTATCGGTTTGTACATTCCTAGTTTATGTTTAACAGGTAGCAACCACTGCCCTTTATA

a     E  I  A  K  H  V  R  I  K  Y  K  L  S  I  V  G  D  G  K  Y  -

      GGTGCAAGGGATCCAGAGACTAAAATATGGAACGGCATGGTTGGGGAACTTGTCTATGGG
 1501 ---------+---------+---------+---------+---------+---------+ 1560
      CCACGTTCCCTAGGTCTCTGATTTTATACCTTGCCGTACCAACCCCTTGAACAGATACCC

a     G  A  R  D  P  E  T  K  I  W  N  G  M  V  G  E  L  V  Y  G  -

      AGAGCTGATATAGCTGTTGCTCCACTCACTATAACATTGGTCCGTGAAGAAGTCATAGAT
 1561 ---------+---------+---------+---------+---------+---------+ 1620
      TCTCGACTATATCGACAACGAGGTGAGTGATATTGTAACCAGGCACTTCTTCAGTATCTA

a     R  A  D  I  A  V  A  P  L  T  I  T  L  V  R  E  E  V  I  D  -
```

FIG. 4G

```
           TTTTCAAAGCCATTAATGAGCCTGGGCATCTCCATCATGATAAAGAAGCCTCAGAAATCA
      1621 ---------+---------+---------+---------+---------+---------+ 1680
           AAAAGTTTCGGTAATTACTCGGACCCGTAGAGGTAGTACTATTTCTTCGGAGTCTTTAGT

a          F  S  K  P  L  M  S  L  G  I  S  I  M  I  K  K  P  Q  K  S  -

           AAACCAGGCGTATTCTCATTTCTGGATCCCCTGGCTTATGAAATCTGGATGTGCATTGTC
      1681 ---------+---------+---------+---------+---------+---------+ 1740
           TTTGGTCCGCATAAGAGTAAAGACCTAGGGGACCGAATACTTTAGACCTACACGTAACAG

a          K  P  G  V  F  S  F  L  D  P  L  A  Y  E  I  W  M  C  I  V  -

           TTTGCTTACATTGGAGTCAGCGTAGTTCTTTTCCTAGTCAGCAGGTTCAGTCCTTATGAA
      1741 ---------+---------+---------+---------+---------+---------+ 1800
           AAACGAATGTAACCTCAGTCGCATCAAGAAAAGGATCAGTCGTCCAAGTCAGGAATACTT

a          F  A  Y  I  G  V  S  V  V  L  F  L  V  S  R  F  S  P  Y  E  -

           TGGCACTTGGAAGACAACAATGAAGAACCTCGTGACCCACAAAGTCCTCCTGATCCTCCA
      1801 ---------+---------+---------+---------+---------+---------+ 1860
           ACCGTGAACCTTCTGTTGTTACTTCTTGGAGCACTGGGTGTTTCAGGAGGACTAGGAGGT

a          W  H  L  E  D  N  N  E  E  P  R  D  P  Q  S  P  P  D  P  P  -

           AATGAATTTGGAATATTTAACAGTCTTTGGTTTTCCTTGGGTGCCTTTATGCAGCAAGGA
      1861 ---------+---------+---------+---------+---------+---------+ 1920
           TTACTTAAACCTTATAAATTGTCAGAAACCAAAAGGAACCCACGGAAATACGTCGTTCCT

a          N  E  F  G  I  F  N  S  L  W  F  S  L  G  A  F  M  Q  Q  G  -
```

FIG. 4H

```
         TGTGATATTTCTCCAAGATCACTCTCCGGGCGCATTGTTGGAGGGGTTTGGTGGTTCTTC
    1921 ---------+---------+---------+---------+---------+---------+ 1980
         ACACTATAAAGAGGTTCTAGTGAGAGGCCCGCGTAACAACCTCCCCAAACCACCAAGAAG

a         C  D  I  S  P  R  S  L  S  G  R  I  V  G  G  V  W  W  F  F  -

         ACCCTGATCATAATTTCTTCCTATACTGCCAATCTCGCTGCTTTCCTGACTGTGGAGAGG
    1981 ---------+---------+---------+---------+---------+---------+ 2040
         TGGGACTAGTATTAAAGAAGGATATGACGGTTAGAGCGACGAAAGGACTGACACCTCTCC

a         T  L  I  I  I  S  S  Y  T  A  N  L  A  A  F  L  T  V  E  R  -

         ATGGTTTCTCCCATAGAGAGTGCTGAAGACTTAGCTAAACAGACTGAAATTGCATATGGG
    2041 ---------+---------+---------+---------+---------+---------+ 2100
         TACCAAAGAGGGTATCTCTCACGACTTCTGAATCGATTTGTCTGACTTTAACGTATACCC

a         M  V  S  P  I  E  S  A  E  D  L  A  K  Q  T  E  I  A  Y  G  -

         ACCCTGGACTCCGGTTCAACAAAAGAATTTTTCAGAAGATCCAAAATTGCTGTGTACGAG
    2101 ---------+---------+---------+---------+---------+---------+ 2160
         TGGGACCTGAGGCCAAGTTGTTTTCTTAAAAAGTCTTCTAGGTTTTAACGACACATGCTC

a         T  L  D  S  G  S  T  K  E  F  F  R  R  S  K  I  A  V  Y  E  -

         AAAATGTGGTCTTACATGAAATCAGCGGAGCCATCTGTGTTTACCAAAACAACAGCAGAC
    2161 ---------+---------+---------+---------+---------+---------+ 2220
         TTTTACACCAGAATGTACTTTAGTCGCCTCGGTAGACACAAATGGTTTTGTTGTCGTCTG

a         K  M  W  S  Y  M  K  S  A  E  P  S  V  F  T  K  T  T  A  D  -
```

FIG. 4I

```
        GGAGTGGCCCGAGTGCGAAAGTCCAAGGGAAAGTTCGCCTTCCTGCTGGAGTCAACCATG
   2221 ---------+---------+---------+---------+---------+---------+ 2280
        CCTCACCGGGCTCACGCTTTCAGGTTCCCTTTCAAGCGGAAGGACGACCTCAGTTGGTAC

a       G  V  A  R  V  R  K  S  K  G  K  F  A  F  L  L  E  S  T  M   -

        AATGAGTACATTGAGCAGAGAAAACCATGTGATACGATGAAAGTTGGTGGAAATCTGGAT
   2281 ---------+---------+---------+---------+---------+---------+ 2340
        TTACTCATGTAACTCGTCTCTTTTGGTACACTATGCTACTTTCAACCACCTTTAGACCTA

a       N  E  Y  I  E  Q  R  K  P  C  D  T  M  K  V  G  G  N  L  D   -

        TCCAAAGGCTATGGTGTGGCAACCCCTAAAGGCTCAGCATTAGGAACGCCTGTAAACCTT
   2341 ---------+---------+---------+---------+---------+---------+ 2400
        AGGTTTCCGATACCACACCGTTGGGGATTTCCGAGTCGTAATCCTTGCGGACATTTGGAA

a       S  K  G  Y  G  V  A  T  P  K  G  S  A  L  G  T  P  V  N  L   -

        GCAGTATTGAAACTCAGTGAACAAGGCATCTTAGACAAGCTGAAAAACAAATGGTGGTAC
   2401 ---------+---------+---------+---------+---------+---------+ 2460
        CGTCATAACTTTGAGTCACTTGTTCCGTAGAATCTGTTCGACTTTTTGTTTACCACCATG

a       A  V  L  K  L  S  E  Q  G  I  L  D  K  L  K  N  K  W  W  Y   -

        GATAAGGGGGAATGTGGAGCCAAGGACTCCGGGAGTAAGGACAAGACCAGCGCTCTGAGC
   2461 ---------+---------+---------+---------+---------+---------+ 2520
        CTATTCCCCCTTACACCTCGGTTCCTGAGGCCCTCATTCCTGTTCTGGTCGCGAGACTCG

a       D  K  G  E  C  G  A  K  D  S  G  S  K  D  K  T  S  A  L  S   -
```

FIG. 4J

```
          CTGAGCAATGTGGCAGGCGTTTTCTATATACTTGTCGGAGGTCTGGGGCTGGCCATGATG
     2521 ---------+---------+---------+---------+---------+---------+ 2580
          GACTCGTTACACCGTCCGCAAAAGATATATGAACAGCCTCCAGACCCCGACCGGTACTAC

a         L  S  N  V  A  G  V  F  Y  I  L  V  G  G  L  G  L  A  M  M   -

                    EcoRI
                    |
          GTGGCTTTGATAGAATTCTGTTACAAATCACGGGCAGAGTCCAAACGCATGAAACTCACA
     2581 ---------+---------+---------+---------+---------+---------+ 2640
          CACCGAAACTATCTTAAGACAATGTTTAGTGCCCGTCTCAGGTTTGCGTACTTTGAGTGT

a         V  A  L  I  E  F  C  Y  K  S  R  A  E  S  K  R  M  K  L  T   -

          AAGAACACCCAAAACTTTAAGCCTGCTCCTGCCACCAACACTCAGAATTATGCTACATAC
     2641 ---------+---------+---------+---------+---------+---------+ 2700
          TTCTTGTGGGTTTTGAAATTCGGACGAGGACGGTGGTTGTGAGTCTTAATACGATGTATG

a         K  N  T  Q  N  F  K  P  A  P  A  T  N  T  Q  N  Y  A  T  Y   -

          AGAGAAGGCTACAACGTGTATGGAACAGAGAGTGTTAAGATCTAGGGATCCCTTCCCACT
     2701 ---------+---------+---------+---------+---------+---------+ 2760
          TCTCTTCCGATGTTGCACATACCTTGTCTCTCACAATTCTAGATCCCTAGGGAAGGGTGA

a         R  E  G  Y  N  V  Y  G  T  E  S  V  K  I  *                 -

          GGAGGCATGTGATGAGAGGAAATCACCGAAAACGTGGCTGCTTCAAGGATCCTGAGCCAG
     2761 ---------+---------+---------+---------+---------+---------+ 2820
          CCTCCGTACACTACTCTCCTTTAGTGGCTTTTGCACCGACGAAGTTCCTAGGACTCGGTC
```

FIG. 4K

```
     ATTTCACTCTCCTTGGTGTCGGGCATGACACGAATATTGCTGATGGTGCAATGACCTTTC
2821 ---------+---------+---------+---------+---------+---------+ 2880
     TAAAGTGAGAGGAACCACAGCCCGTACTGTGCTTATAACGACTACCACGTTACTGGAAAG

     AATAGGAAAAACTGATTTTTTTTTTCCTTCAGTGCCTTATGGAACACTCTGAGACTCGCG
2881 ---------+---------+---------+---------+---------+---------+ 2940
     TTATCCTTTTTGACTAAAAAAAAAAGGAAGTCACGGAATACCTTGTGAGACTCTGAGCGC

     ACAATGCAAACCATCATTGAAATCTTTTTGCTTTGCTTGAAAAAAAATAATTAAAATAAA
2941 ---------+---------+---------+---------+---------+---------+ 3000
     TGTTACGTTTGGTAGTAACTTTAGAAAAACGAAACGAACTTTTTTTTATTAATTTTATTT

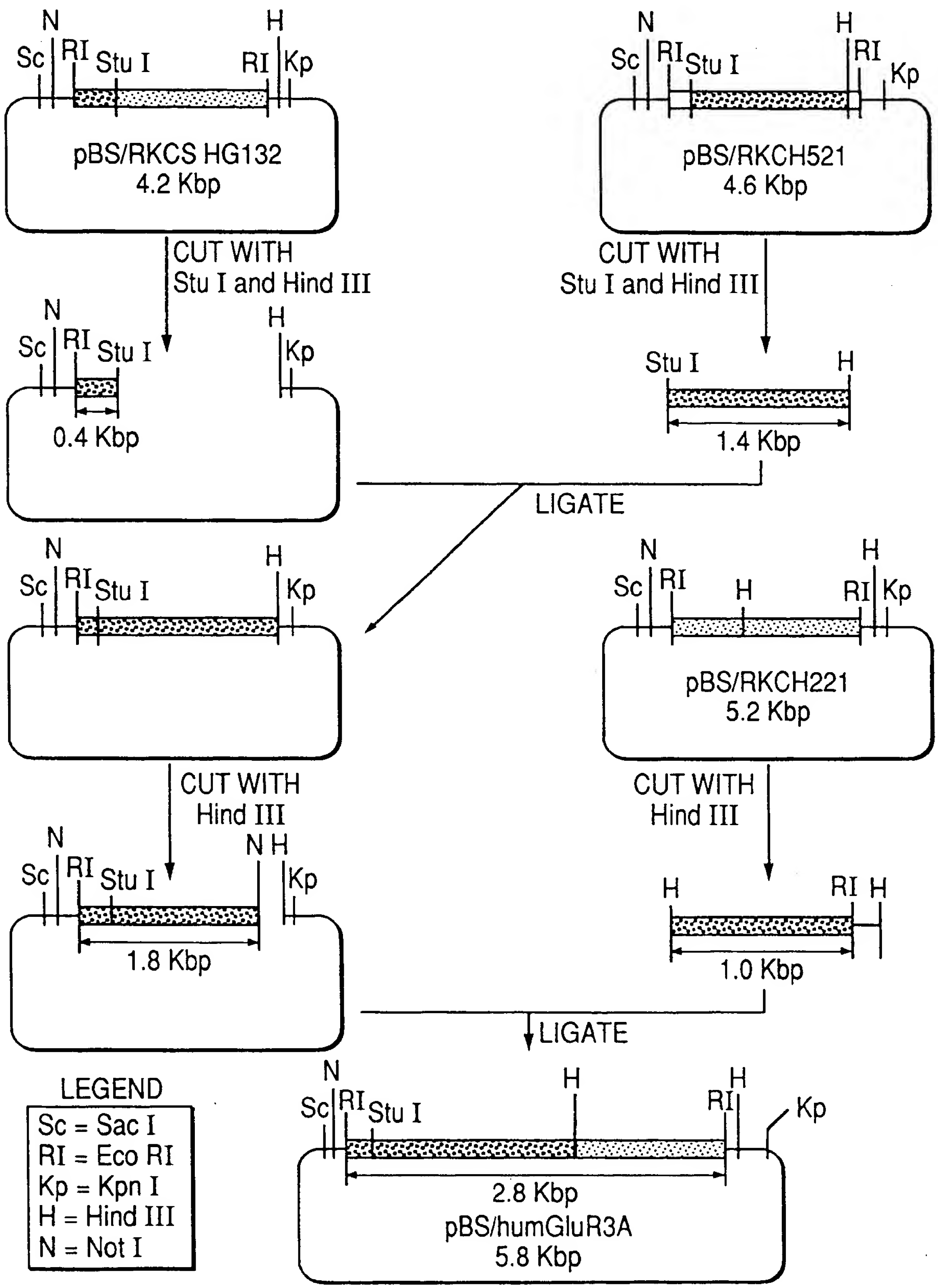
     AACCAACAAAAATGGACATGCATCAAACCCTTGATGTATTAATATTTATTATAGTTTTCA
3001 ---------+---------+---------+---------+---------+---------+ 3060
     TTGGTTGTTTTTACCTGTACGTAGTTTGGGAACTACATAATTATAAATAATATCAAAAGT

     TTAGGAATTC
3061 ---------+ 3070
     AATCCTTAAG
```

FIG. 5

```
GluR3A  ..GSALGNAVNLAVLKLNEQGLLDKLKNKWWYDKGECGSGGGDSKDKT..
        IIIIIII  IIIIIIII III IIIIIIIIIIIIIIII     IIIIIII
GluR3B  ..GSALGTPVNLAVLKLSEQGILDKLKNKWWYDKGECGAKDSGSKDKT..
```

FIG. 6



N
H
Sc
RI
Stu I
RI
Kp
pBS/RKCS HG132
4.2 Kbp
N
H
Sc
RI
Stu I
RI
Kp
pBS/RKCH521
4.6 Kbp
CUT WITH
Stu I and Hind III
CUT WITH
Stu I and Hind III
N
Sc
RI
Stu I
H
Kp
0.4 Kbp
Stu I
H
1.4 Kbp
LIGATE
N
Sc
RI
Stu I
H
Kp
N
H
Sc
RI
H
RI
Kp
pBS/RKCH221
5.2 Kbp
CUT WITH
Hind III
CUT WITH
Hind III
N
Sc
RI
Stu I
N H
Kp
1.8 Kbp
H
RI H
1.0 Kbp
LIGATE
LEGEND
Sc = Sac I
RI = Eco RI
Kp = Kpn I
H = Hind III
N = Not I
N
Sc
RI
Stu I
H
H
RI
Kp
2.8 Kbp
pBS/humGluR3A
5.8 Kbp

FIG. 7

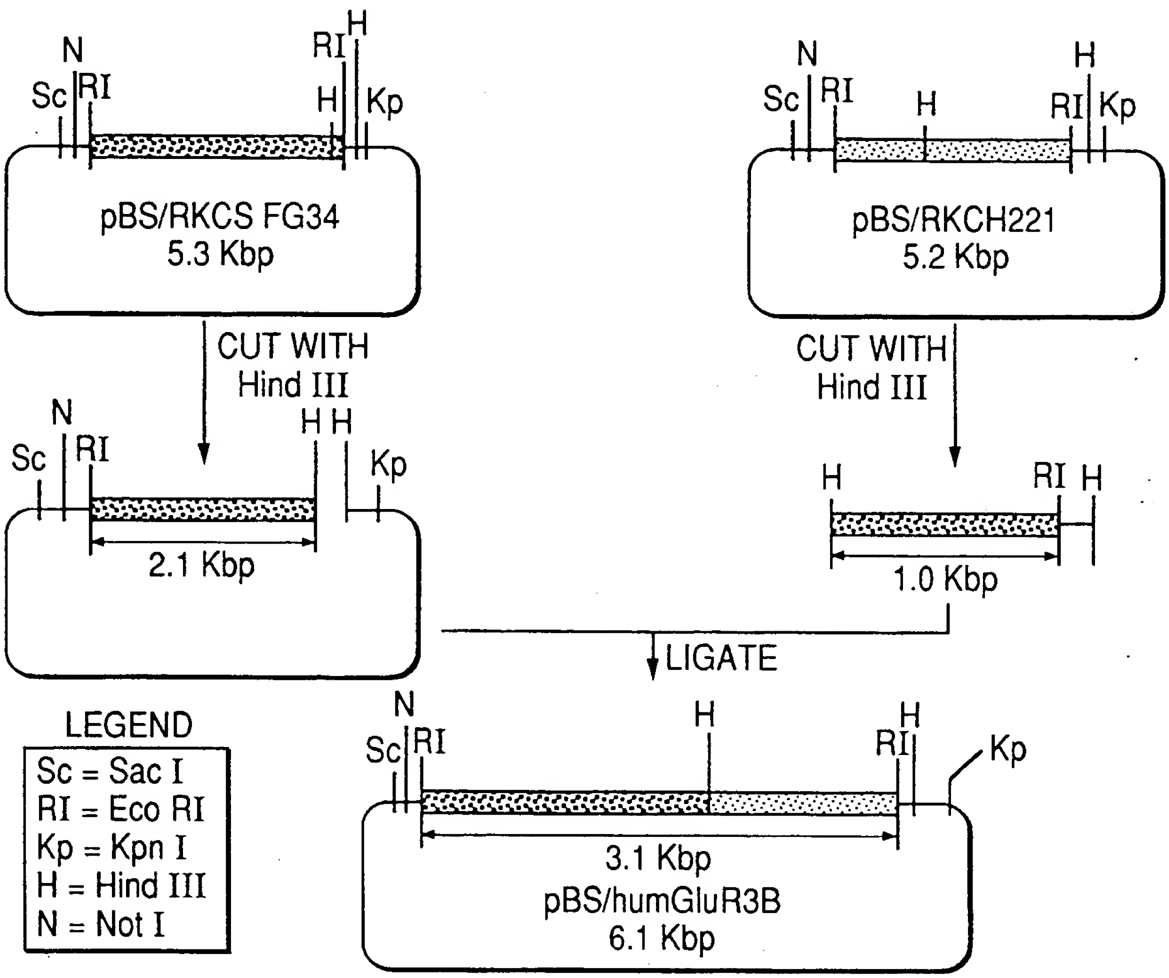
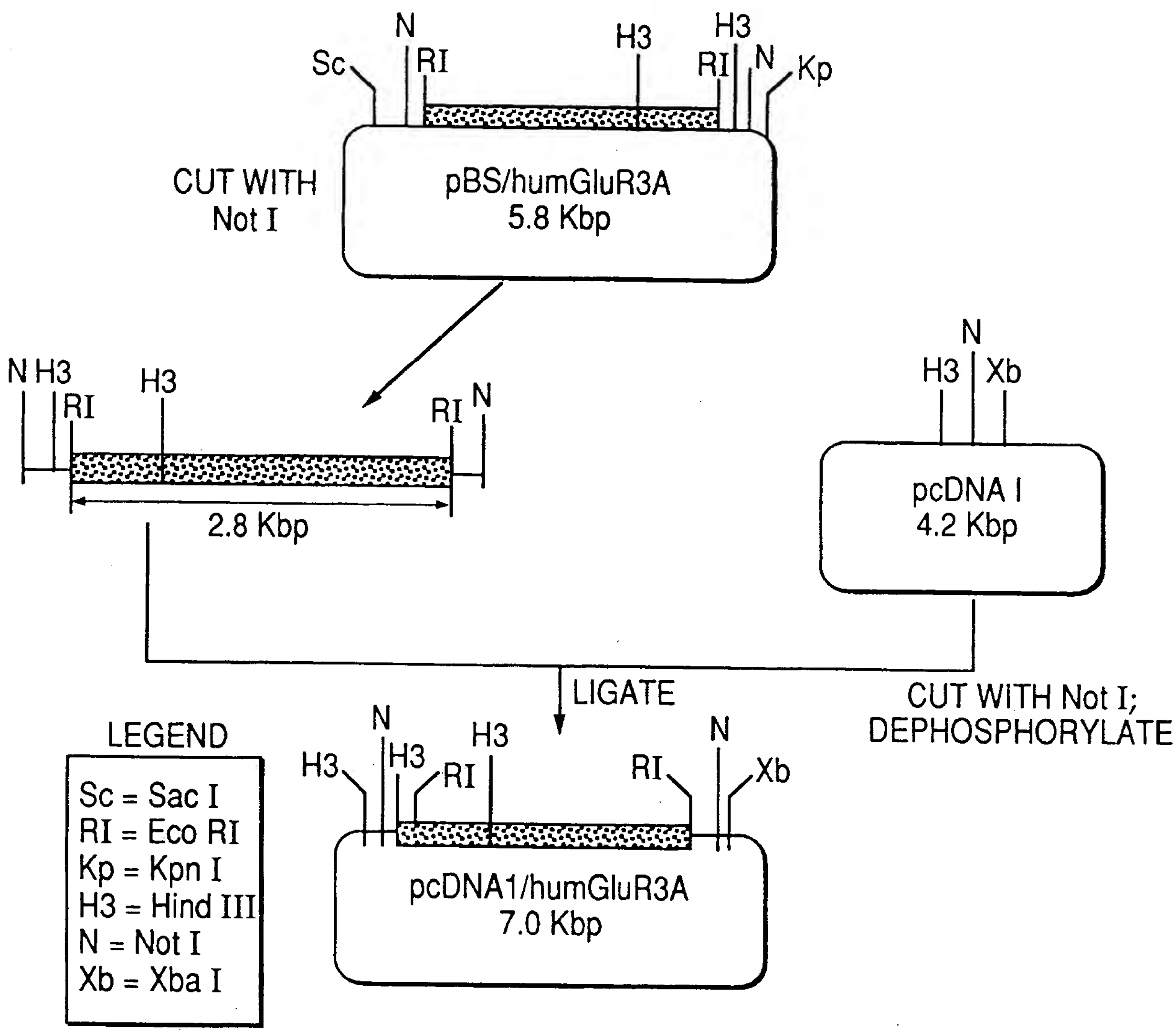

N
Sc
RI
RI
H
H
Kp
pBS/RKCS FG34
5.3 Kbp
N
Sc
RI
H
H
RI
Kp
pBS/RKCH221
5.2 Kbp
CUT WITH
Hind III
CUT WITH
Hind III
N
Sc
RI
H H
Kp
2.1 Kbp
H
RI H
1.0 Kbp
LIGATE
LEGEND
Sc = Sac I
RI = Eco RI
Kp = Kpn I
H = Hind III
N = Not I
N
Sc
RI
H
H
RI
Kp
3.1 Kbp
pBS/humGluR3B
6.1 Kbp

FIG. 8



N
Sc
RI
H3
H3
RI
N
Kp
CUT WITH
Not I
pBS/humGluR3A
5.8 Kbp
N H3
RI
H3
RI
N
2.8 Kbp
N
H3
Xb
pcDNA I
4.2 Kbp
LIGATE
CUT WITH Not I;
DEPHOSPHORYLATE
LEGEND
Sc = Sac I
RI = Eco RI
Kp = Kpn I
H3 = Hind III
N = Not I
Xb = Xba I
N
H3
H3
RI
H3
N
RI
Xb
pcDNA1/humGluR3A
7.0 Kbp

FIG. 9

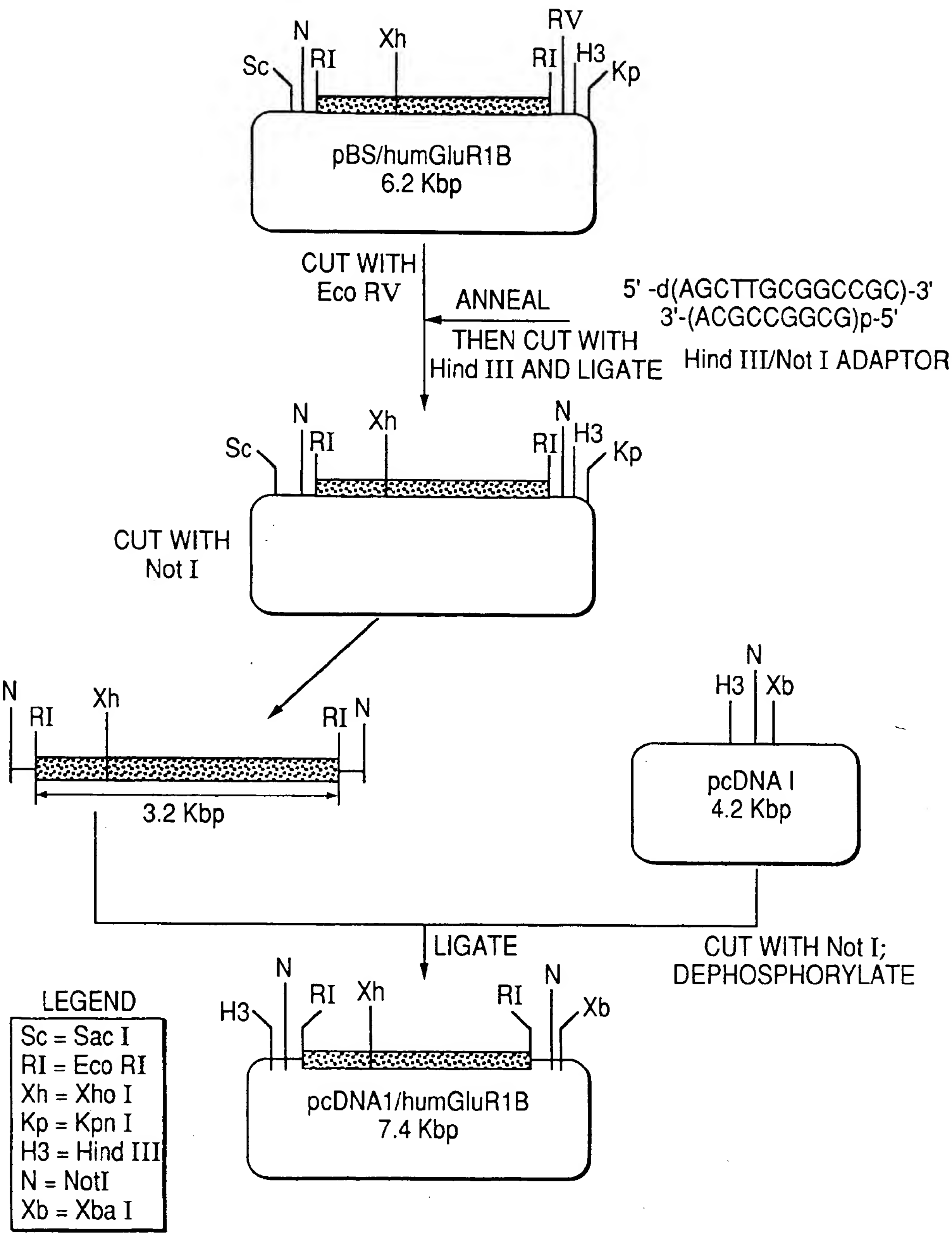

N
Xh
RV
Sc
RI
RI
H3
Kp
pBS/humGluR1B
6.2 Kbp
CUT WITH
Eco RV
ANNEAL
5' -d(AGCTTGCGGCCGC)-3'
3'-(ACGCCGGCG)p-5'
THEN CUT WITH
Hind III AND LIGATE
Hind III/Not I ADAPTOR
N
Xh
N
Sc
RI
RI
H3
Kp
CUT WITH
Not I
N
RI
Xh
RI
N
3.2 Kbp
N
H3
Xb
pcDNA I
4.2 Kbp
LIGATE
CUT WITH Not I;
DEPHOSPHORYLATE
N
RI
Xh
RI
N
H3
Xb
pcDNA1/humGluR1B
7.4 Kbp
LEGEND
Sc = Sac I
RI = Eco RI
Xh = Xho I
Kp = Kpn I
H3 = Hind III
N = NotI
Xb = Xba I

FIG. 10

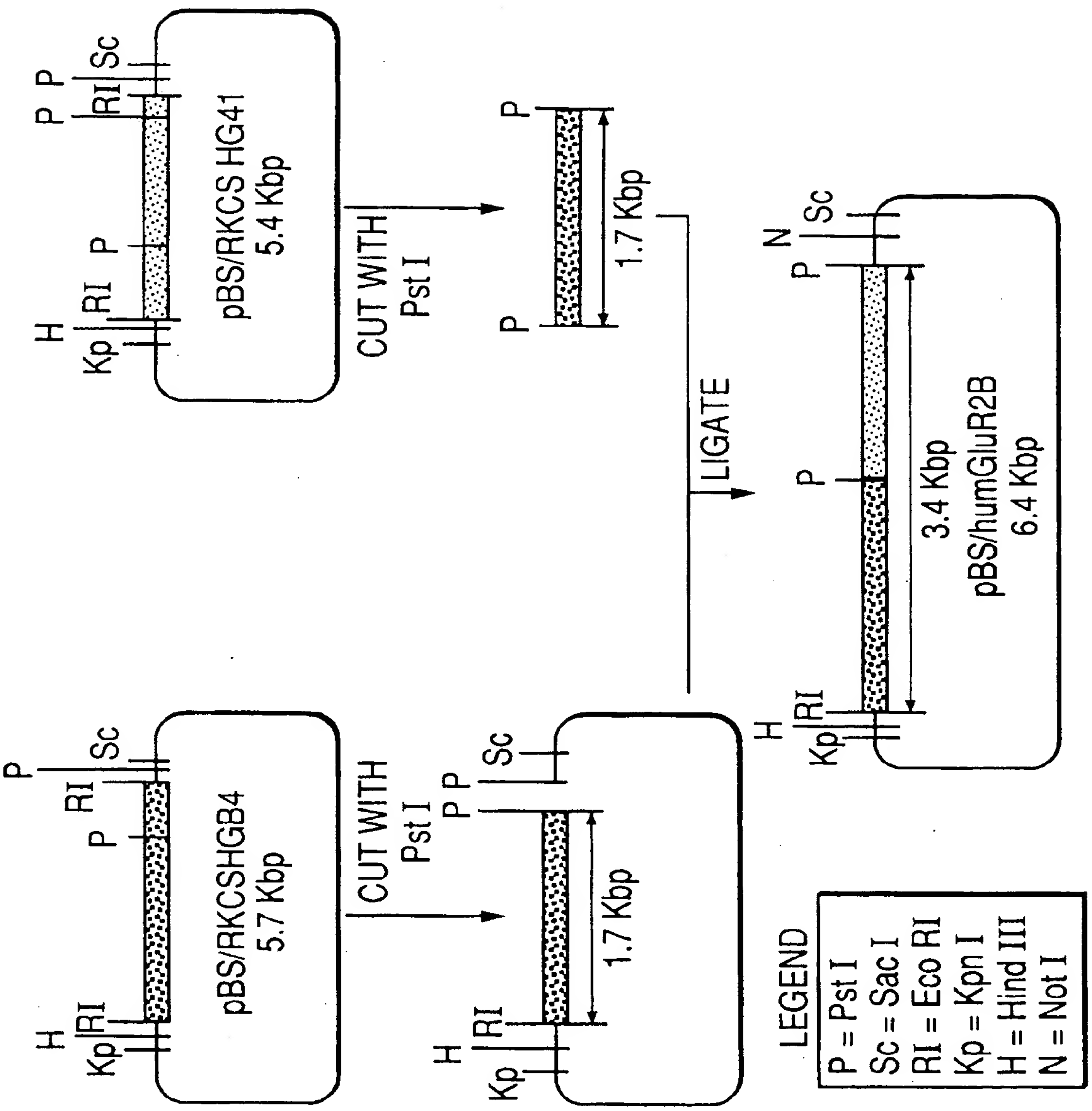

pBS/RKCSHGB4
5.7 Kbp
pBS/RKCS HG41
5.4 Kbp
CUT WITH
Pst I
1.7 Kbp
LIGATE
3.4 Kbp
pBS/humGluR2B
6.4 Kbp
H
Kp
RI
P
Sc
N
LEGEND
P = Pst I
Sc = Sac I
RI = Eco RI
Kp = Kpn I
H = Hind III
N = Not I

FIG. 11

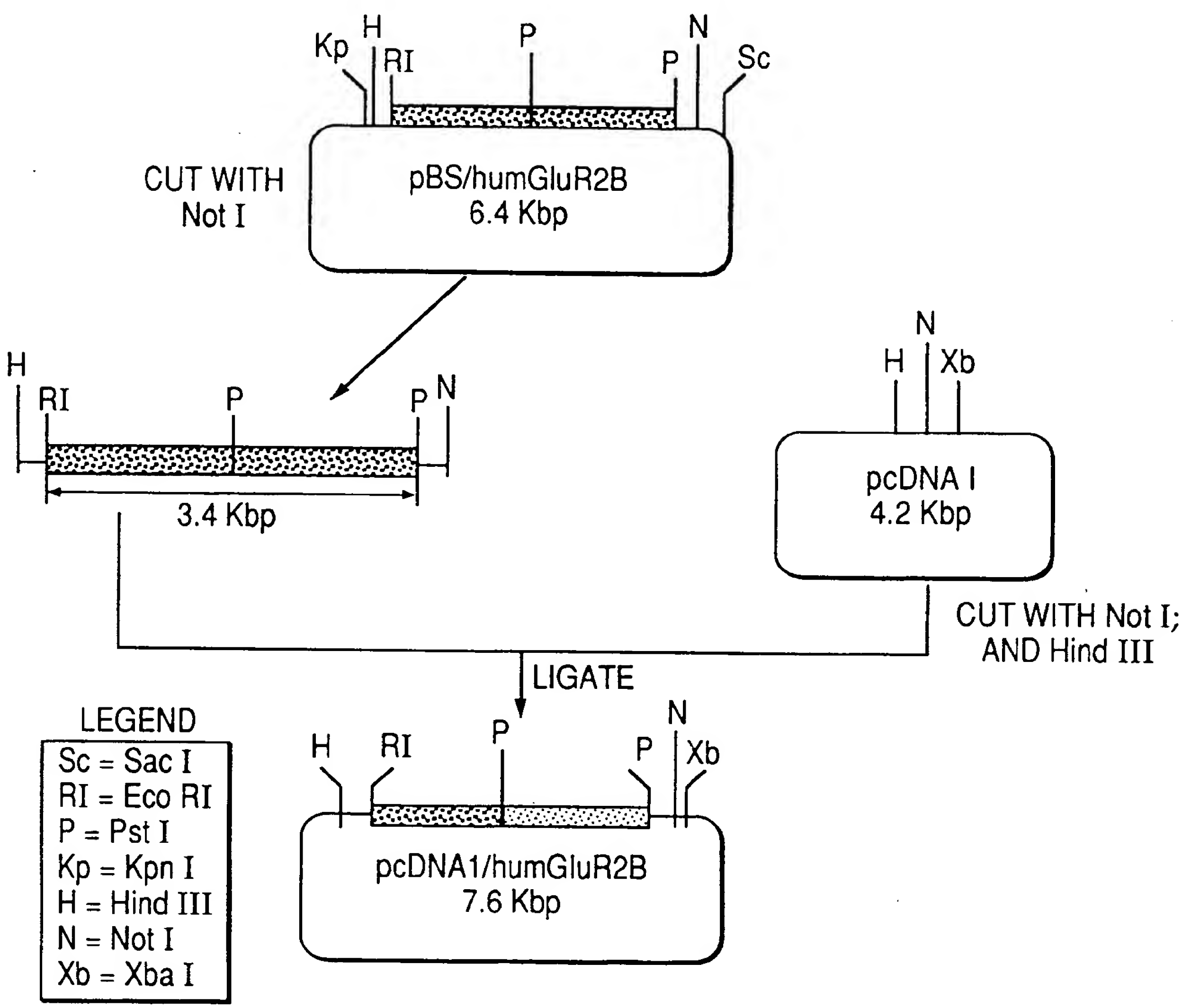

Kp
H
RI
P
P
N
Sc
CUT WITH
Not I
pBS/humGluR2B
6.4 Kbp
H
RI
P
P
N
3.4 Kbp
N
H
Xb
pcDNA I
4.2 Kbp
CUT WITH Not I;
AND Hind III
LIGATE
LEGEND
Sc = Sac I
RI = Eco RI
P = Pst I
Kp = Kpn I
H = Hind III
N = Not I
Xb = Xba I
H
RI
P
P
N
Xb
pcDNA1/humGluR2B
7.6 Kbp

FIG. 12

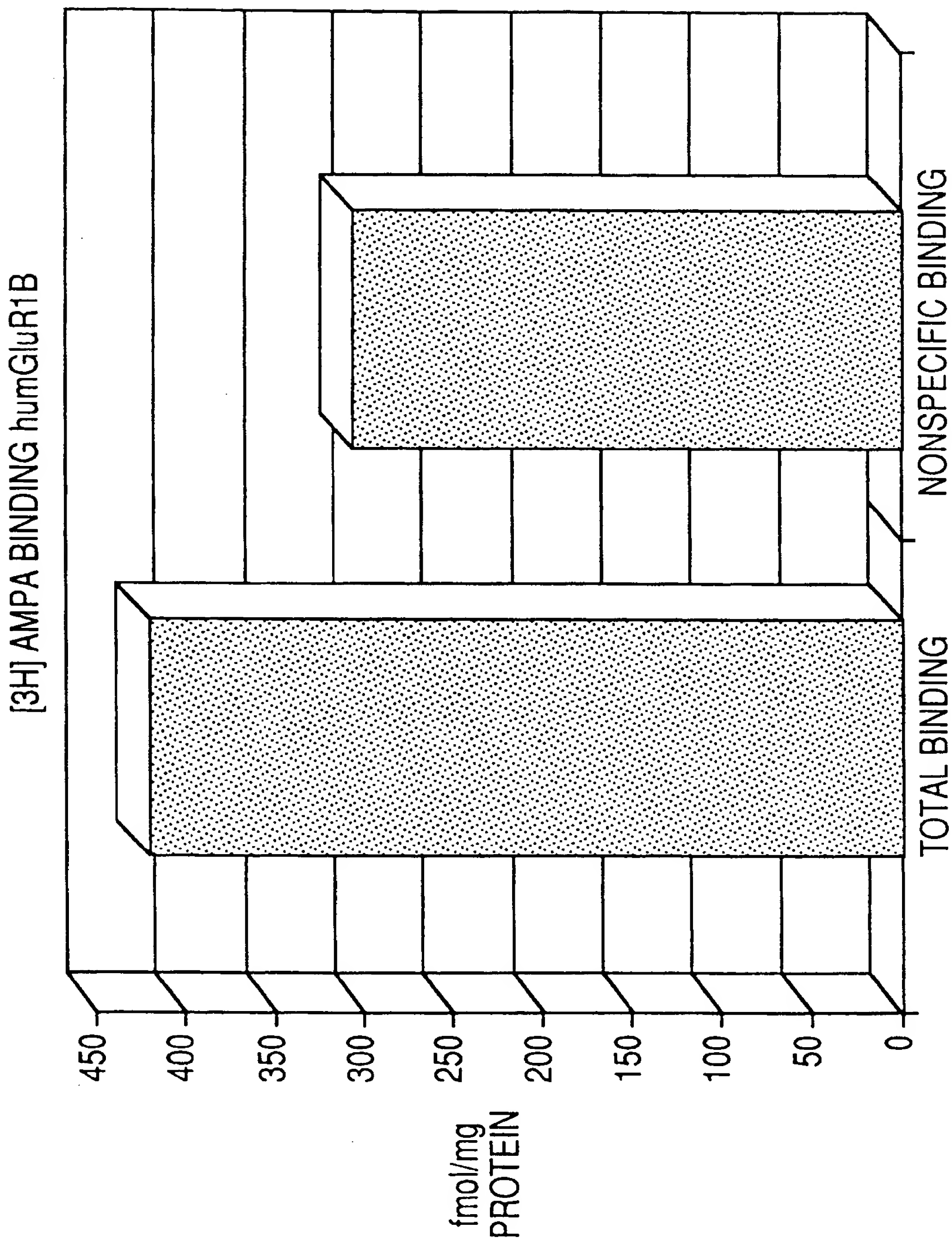

[3H] AMPA BINDING humGluR1B
fmol/mg
PROTEIN
450
400
350
300
250
200
150
100
50
0
TOTAL BINDING
NONSPECIFIC BINDING

FIG. 13

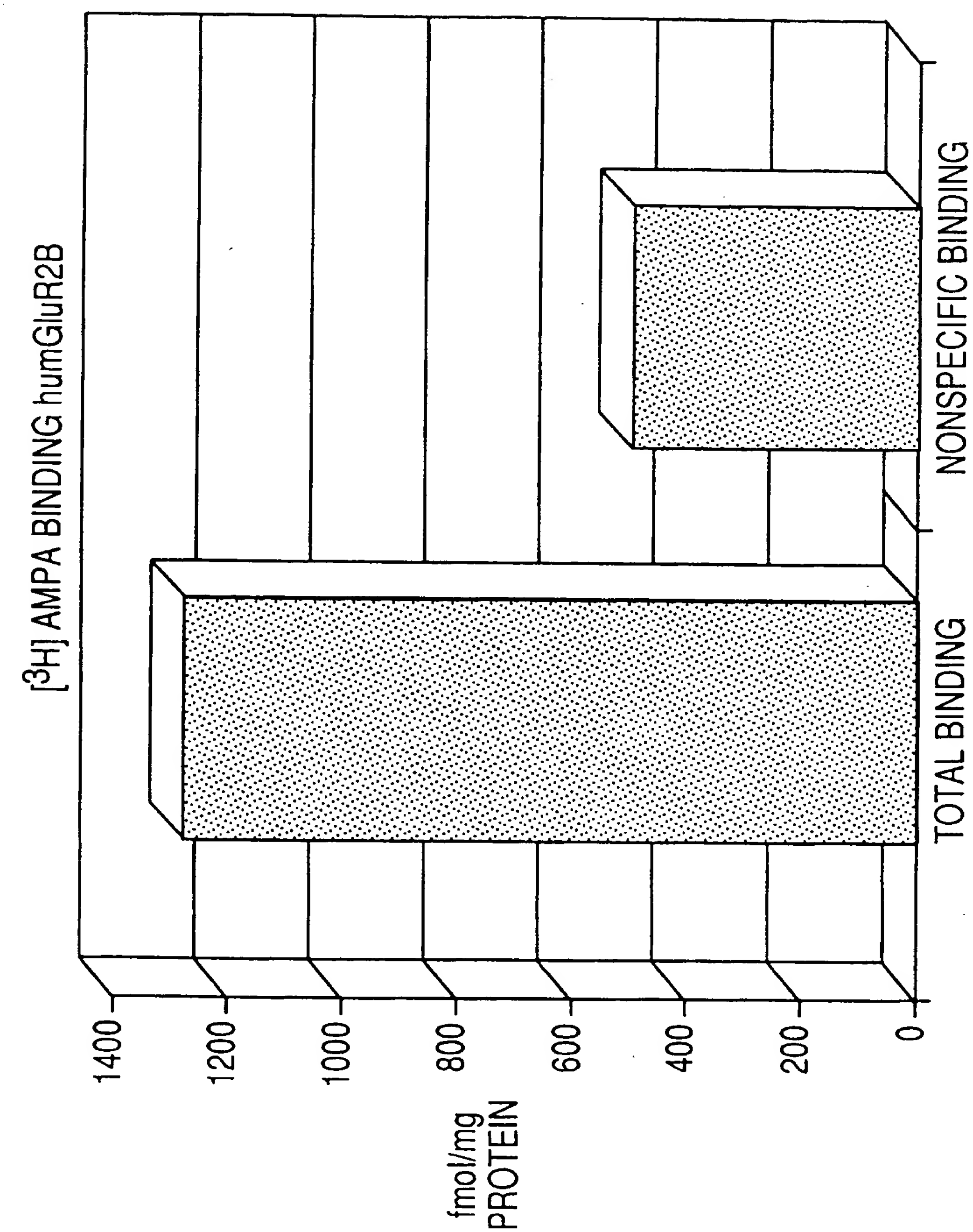
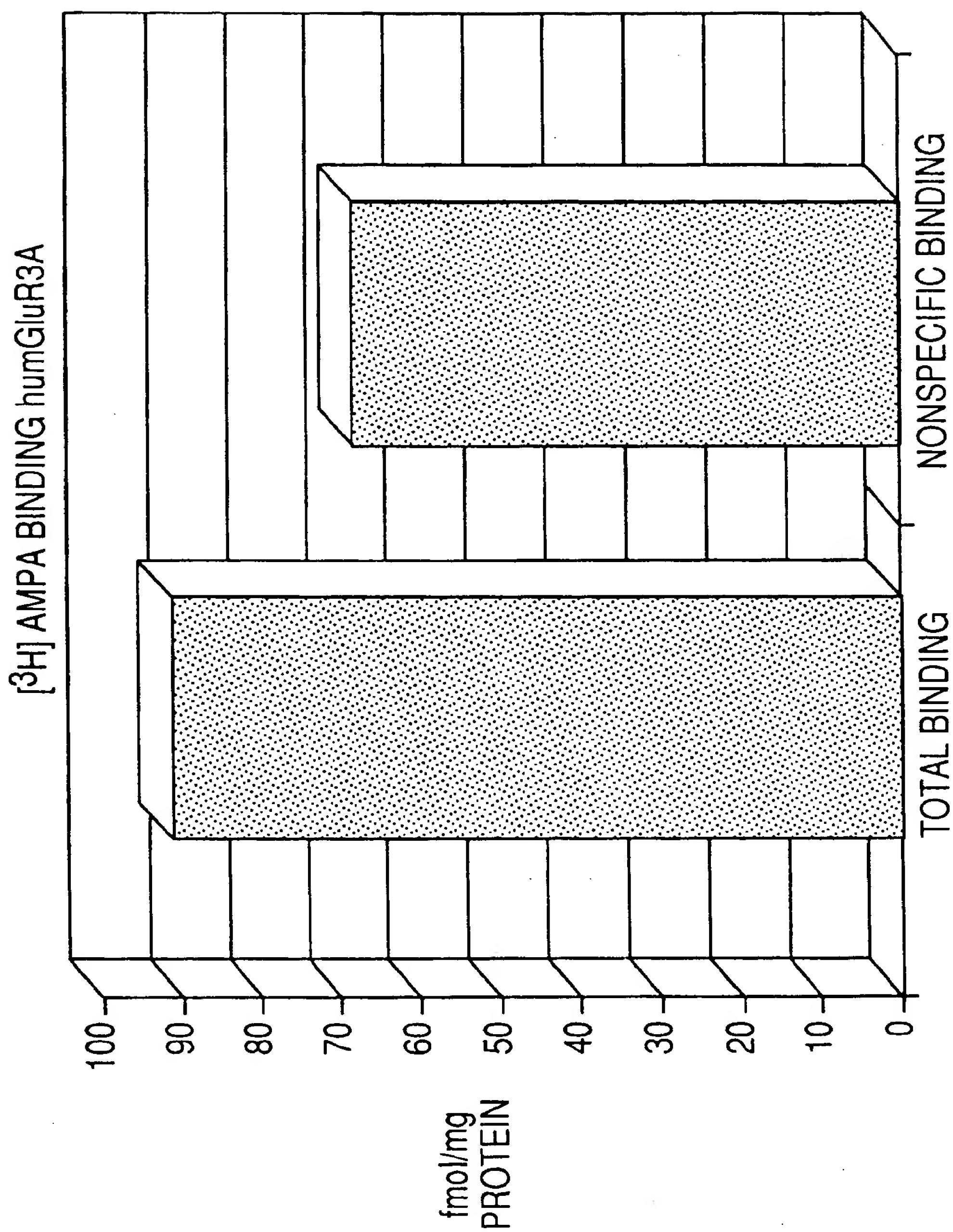

[3H] AMPA BINDING humGluR2B
fmol/mg PROTEIN
1400
1200
1000
800
600
400
200
0
TOTAL BINDING
NONSPECIFIC BINDING

FIG. 14



[3H] AMPA BINDING humGluR3A
fmol/mg PROTEIN
100
90
80
70
60
50
40
30
20
10
0
TOTAL BINDING
NONSPECIFIC BINDING

FIG. 15A

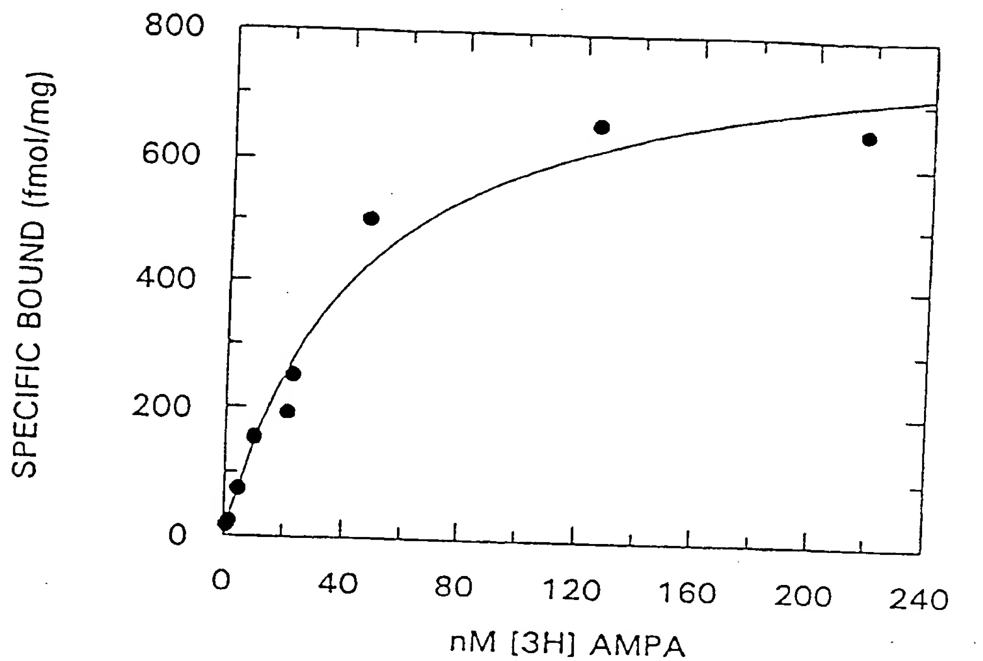


FIG. 15B

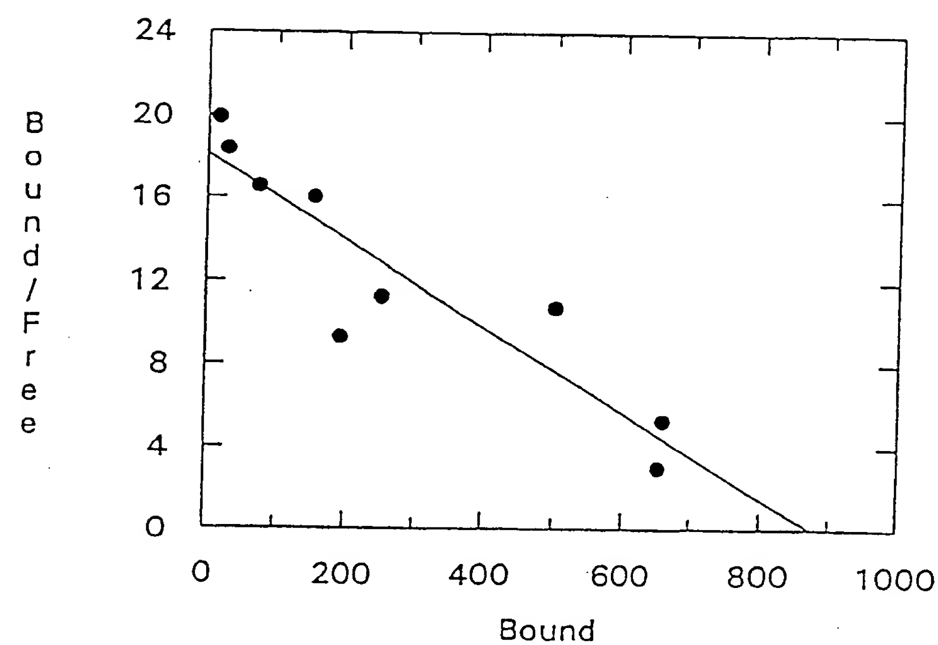


FIG. 16A

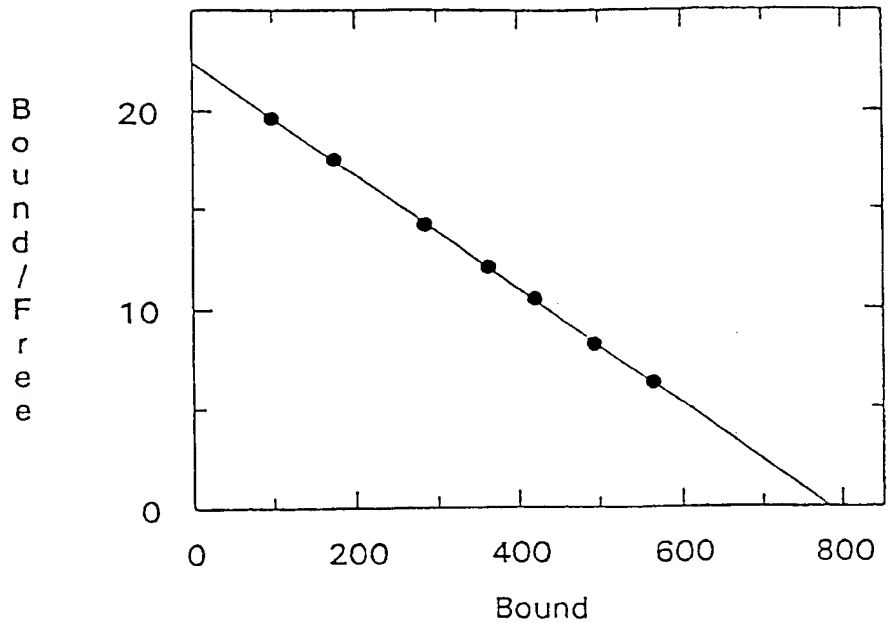


FIG. 16B

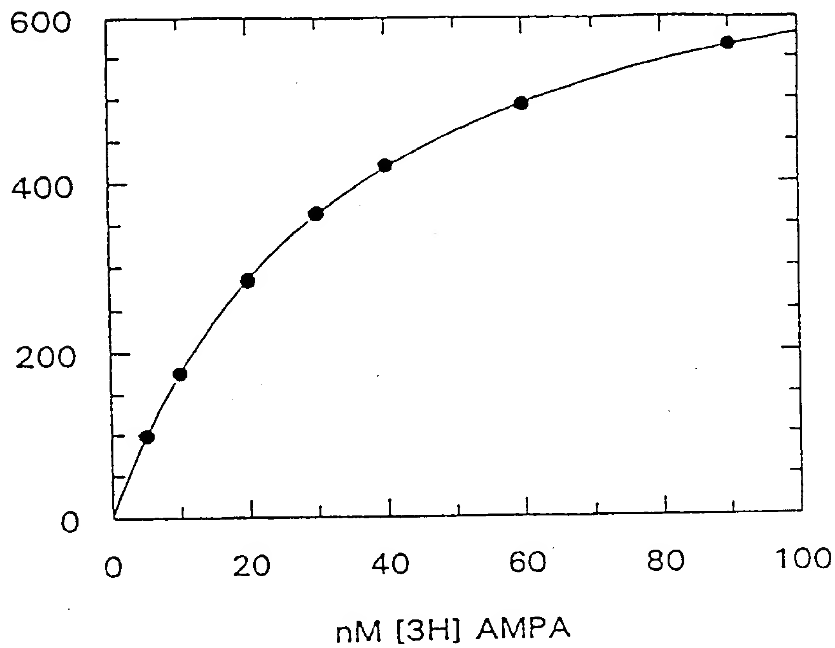
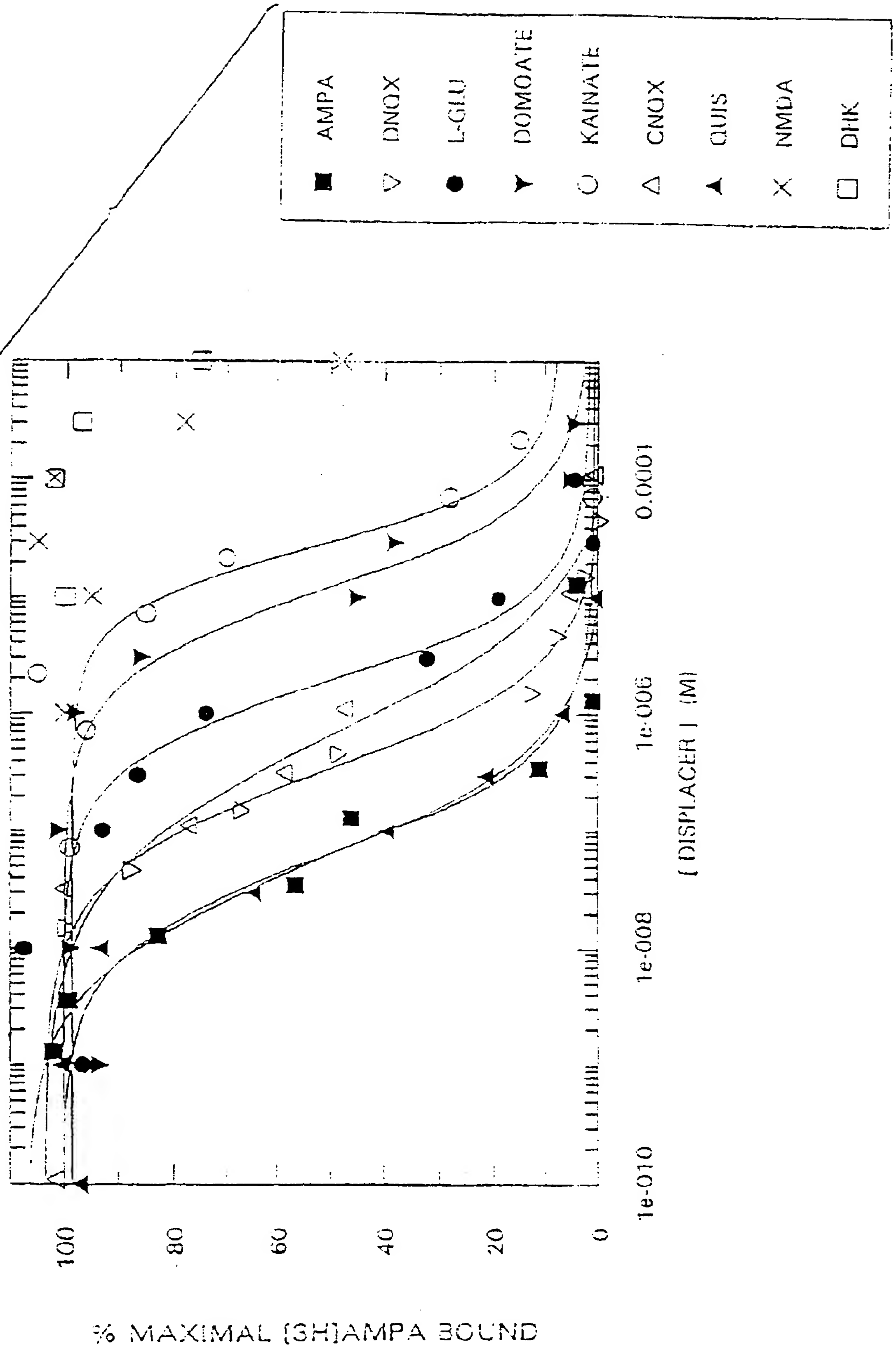


FIG. 17


AMPA
DNQX
L-GLU
DOMOATE
KAINATE
CNQX
QUIS
NMDA
DHK
100
80
60
40
20
0
1e-010
1e-008
1e-006
0.0001
[DISPLACER] (M)
% MAXIMAL [3H]AMPA BOUND

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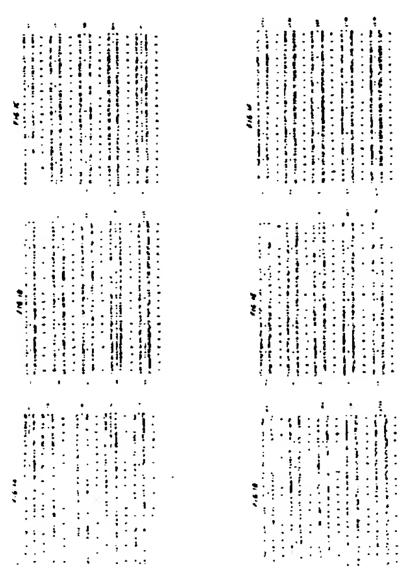
(54) Amino-hydroxy-methyl-isoxazole-propionate binding human glutamate receptors.

(57) Described herein are isolated polynucleotides which code for a family of AMPA-type human CNS receptors. The receptors are characterized structurally and the construction and use of cell lines expressing these receptors are disclosed.



EP 0 574 257 A3

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EUROPEAN SEARCH REPORT

Application Number
EP 93 30 4500

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.5)
Y	WO-A-91 06648 (THE SALK INSITUTE FOR BIOLOGICAL STUDIES) * the whole document * ---	1-18	C07K15/00 C12N15/12 C12N5/08 C12P21/02 C12P21/08 G01N33/48
Y,D	SCIENCE., vol.249, no.4968, 1990, LANCASTER, PA US pages 556 - 560 KEINÄNEN K. ET AL 'A family of ampa...' * the whole document * ---	1-18	
A	DNA SEQUENCE-J.DNA SEQUENCING AND MAPPING, vol.2, 1992, UK pages 211 - 218 POTIER M.C. ET AL. 'The glutamate receptor...' * the whole document * -----	1-12	
			TECHNICAL FIELDS SEARCHED (Int.Cl.5)
			C07K

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
BERLIN	23 November 1994	Gurdjian, D

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